PSITTACOSIS

Diagnosis Epidemiology and Control

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Diagnosis, Epidemiology and Control

Edited by F R Beaudette

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Foreword

This book is a record of the proceedings of a symposium on psittacosis held in New York City on May 5th and 6th 1953 under the auspices of the New Jersey Agricultural Experiment Station Rutgers University The meeting was made possible through the generosity of Mr Gustav and Mr Max Stern and Mr William Odenwald of the Hartz Mountain Products Company of New York.

Since Hartz is the largest manufacturer of products for pets and is also the largest importer and distributor of birds it is understandable that the owners would have some knowledge of psittacosis including the information that all human psittacosis does not necessarily originate from contact with birds of the parrot family The owners desire to acquire an up to date knowledge of all aspects of the disease and make this available to persons in academic and public health positions seemed most easily accomplished by bringing authorities in the field together to present their findings and views

There was also the feeling that much could still be learned about psittacosis and it was anticipated that the meeting would raise interesting questions and stimulate investigators to search for the answers And if there appeared to be a lack of financial support for such research our host let it be known that some help could be supplied

Here it is necessary for the editor to account for his connection with the project This began years ago incident to an outbreak of pox in a population of canaries which was brought to our attention at Rutgers by Mr Gustav Stern As a result of the studies made at least a partial solution to the problem was developed

Thus when it was desired to arrange for the symposium the editor more or less inherited the responsibility through his position as Poultry Pathologist at Rutgers University and the New Jersey Agricultural Experiment Station

It should therefore be evident from the outset that the editor has no special knowledge of psittacosis or ornithosis infections As a matter of fact this lack of knowledge has been an advantage in recognizing what information is needed by the uninitiated and how the contributions dealing with the various aspects of the disease might best be arranged

Thus there is presented a brief history of the disease for the benefit of the beginner with a general survey of the agents that make up the psittacosis viruses an account of their isolation and identification by serologic methods and a discussion of the clinical aspects of the disease its pathology and diagnosis by virus isolation and other means

Papers concerning the disease in turkeys chickens wild and domestic pigeons ducks sea birds and birds in zoological collections make it abundantly evident that psittacine birds are not the sole sources of human infection Nor is it possible to exclude mammals as reservoirs Moreover the Louisiana pneumonitis outbreak caused by an agent of the psittacosis group certainly indicates that man suffering from this type of disease is a greater source of infection than heretofore realized The results of the investigation made in Florida would suggest that overemphasis had been placed on psittacine birds however the same factors in a different region might have created a different picture

In any event we have reached a point of understanding that quarantine measures directed entirely to traffic in psittacine birds are inadequate There is some basis for the suspicion that these regulations will have to be drastically modified when the full host range of the psittacosis viruses is known This is as it has always been for the fact that a disease is named after a species or locality is often misleading eastern equine encephalomyelitis as an example did not remain restricted to the east or to the horse

Since the holding of the meeting in New York turkeys have been responsible for a large number of human cases so that now

psittacosis has to be considered as an occupational disease of workers in dressing plants

There can be no doubt that cases of human psittacosis will continue to develop but fortunately antibiotic therapy has been eminently successful And it is reasonable to expect that the outstanding investigators engaged in this field will continue not only to extend our knowledge of the animal reservoir of the psittacosis viruses but also to perfect the methods of diagnosis and treatment Meantime the practicing physician interested in unusual viral infections may find this book a ready reference It should also prove useful to virologists who are concerned with little known viral agents and to whom suspect material may be sent for diagnosis

It can be recorded that within the year the Hartz Mountain Products Company has supplied funds for a project on Experimental Psittacosis Studies on Birds at the University of Texas Medical Branch Galveston Texas two projects on Serological Diagnosis of Psittacosis and "Field Diagnosis of Psittacosis at the University of Pennsylvania Philadelphia and a project on the "Biological and Chemical Properties of Antibiotics Inhibitory to the Chlamydozoacea with Particular Reference to the Psittacosis Virus at the Institute of Microbiology Rutgers University New Brunswick New Jersey A fifth project in part involving therapy and immunization is being organized at the University of California It is to be hoped that the completion of these projects will supply much needed information and in particular a simplified and yet reliable method of diagnosis that can be used by poultry pathologists to whom large numbers of potential hosts are brought for routine diagnosis

F R BEAUDETTE

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PSITTACOSIS

Diagnosis Epidemiology and Control

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Chapter 14

PSITTACOSIS

Diagnosis Epidemiology and Control

C E VAN ROOYEN, M D

1

The Early History of Psittacosis

FROM 1879 to 1928 repeated outbreaks of illness associated with parrots were observed in certain cities of Germany France Switzerland England and the United States

The disease is said to have been described first as a clinical entity in Europe by Juergensen in 1874 and subsequently by Ritter in 1880 in Switzerland and in 1884 by Wagner who referred to the condition as pneumotyphus Following the large outbreak in Paris in 1892 Morange (1895) studied cases of illness and proposed the name psittacosis for the condition During this same epidemic Nocard (1893) isolated a Gram negative paracolon bacillus from affected birds and for some 38 years Nocard s bacillus was believed to be the causal organism of the malady

From a study of early epidemics it would seem that the circumstances associated with human cases were strikingly similar Trouble usually started with the arrival of large numbers of parrots and other exotic birds following their shipment into Europe from South America The survival rate of these creatures during the transatlantic voyage was invariably low and many are reported to have died After arrival at the seaport towns on the Continent of Europe the birds were promptly distributed without preliminary quarantining to prospective owners situated far and wide over the countryside

Subsequent events revealed that many of the new owners lavished all the care and affection of which they were capable upon their recently acquired pets with the result that the opportunities

for massive transfer of virus from bird to man were doubtless very considerable

The literature contains repeated reference to illness affecting bird fanciers who practiced the objectionable habit of mouth to beak feeding without knowledge of the risks entailed

The tendency to house parrots in domestic living quarters further aggravated the risks of human infection It is also likely that sick and ailing birds received an extra measure of handling and atten tion from their well meaning but unsuspecting owners and so cre- ated a much greater danger to members of the household

The 1892 epidemic in Paris started as an epizootic among a col ony of 500 parrots imported from Buenos Aires by two French animal dealers Both men themselves became infected from the parrots as did those who had purchased the birds The new owners in turn infected their friends relatives and a doctor in attendance upon one of the sick Altogether 51 persons were affected in this epidemic and 16 died (Nocard 1893 Morange 1895)

In 1898 according to a Berlin newspaper report the disease broke out among the visitors to the annual exhibition of the Berlin Union of Canary Fanciers following which five or six people be came seriously ill and three died in agony (Roubakine 1930)

The 1909 epidemic at Zulpich on the Rhine near Cologne origi nated from a single parrot whose owner died of psittacosis The bird however was alive at the time of its master s death and gave rise to further cases by infecting the mourners who had assembled at the deceased s house to attend his funeral ceremony (Bachem Selter and Finkler 1910) A repetition of this incident occurred in New York in 1930 when Rabinowitz and Livingstone (1932) re- ported an outbreak of five cases among the mourners at a Jewish funeral who had congregated at a home in which two parakeets were kept In another epidemic a German professor who brought back four birds to Berlin after a visit to the Upper Amazon in Brazil contracted the disease along with six members of his family and a friend who had visited them (Elkeles 1930)

Kerschensteiner (1930) recorded a somewhat similar outbreak of psittacosis affecting a family of six who lived in Munich and became infected from a parrot they had purchased at a bargain sale Grunwald and Meyer (1930) reported a case of infection ac-

quired by a veterinary surgeon who had worked in close proximity to a parrot Schmid (1931) described an epidemic resembling psittacosis in a veterinary hospital and Maragliano (1897) quoted a case affecting a sailor who kept a bird Finally Horder and Gow (1930) mention the case of a woman who contracted the disease through merely shaking hands with the owner of a sick parrot the patient never saw the bird at any time

An early account of psittacosis in the United States has been provided by Vickery and Richardson (1904) who described a family outbreak affecting a man his wife and domestic helper who acquired infection following the purchase of a Green Amazon parrot in New York

Later in 1917 McClintock reported a group of cases occurring in a large department store at Wilkes Barre Pennsylvania Here several sick birds were received by the store and ultimately sold to purchasers The epidemiologic details are well illustrated in the following excerpt from McClintock (1917)

"In about ten days the sick list of the store employees began to grow At about the same time many peculiar cases of illness appeared in the practices of physicians in Wilkes Barre and the surrounding towns The patients usually gave clear history of contact with the sick parrots either at the store or at the home Some people touched the parrots but in the majority of instances the inspiration of the air of this zone was the common factor There was a number of cases in which the disease in its epidemic form must have been contracted from sick parrots after the birds had been taken to the homes"

The year 1929 marked a turning point in the revival of interest concerning the etiology of human psittacosis Over 100 human beings developed infections during July August and September in Alta Gracia Tucumán and Córdoba in the Argentine Republic (Barros 1929) Further afield fresh curiosity was aroused in Germany the United States and Great Britain and an efficiently directed program of scientific research was launched to ascertain the validity of Nocard s claim to have isolated the causal organism of the disease

The subsequent advances provide a good illustration of the value of the reinvestigation of older problems with the aid of newer

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and Orders 1930 No 299) under the Public Health Act of 1875 The word parrot was made applicable to any bird of the group known as Psittaciformes Only birds consigned to medical research laboratories or zoological gardens were exempt from such regulations

Similar laws were enacted in many of the countries of Europe the United States South Africa New Zealand and elsewhere Where introduced these measures had the desired effect for with them the trade in exotic birds virtually ceased and human cases of psittacosis almost disappeared

No account of early work on psittacosis would be complete without some reference to the many laboratory infections which were acquired during the conduct of investigations Rivers *et al* (1930) reported 16 cases with five deaths at the laboratories of Dr Krumwiede *et al* (1930) in the New York City Department of Health A total of 66 accidental infections with seven deaths was reported by Sulkin and Pike (1949)

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tools and in the light of more modern developments In short it may be said that the discovery of psittacosis virus in 1930 was an outcome of progress in the field of virology which occurred in the period intervening between the description of Nocard s bacillus in 1892 and the year 1930

The events which followed in quick succession in the year 1930 are still familiar to many of us Levinthal (1930) in Germany, and Bedson and Western (1930) and Gordon (1930) in London successfully transmitted the virus from man to both the parrot and the mouse using Berkefeld filtrates of infective tissues Levinthal (1930a b c 1935) also described the occurrence of specific virus elementary bodies in the endothelial cells of inoculated animals Likewise Coles (1930) the English general practitioner working as a free lance amateur microscopist at his country home in Bournemouth England also found the virus in infected mouse tissue Material for pathologic study was supplied to Coles through the courtesy of Professor S P Bedson of the London Hospital and by Dr Mervyn Gordon of St Bartholomew s Hospital London (Bedson and Western 1930 Gordon 1930 and Bedson, Western and Simpson 1930)

Simultaneously independent studies in the United States by Krumwiede McGrath and Oldenbusch (1930) also established the virus etiology of human psittacosis beyond doubt Elementary bodies similar to those described by Levinthal (1930a) and Coles (1930) were also found by Lillie (1930) who proposed the name *Rickettsia psittaci* for them

Spurred by the success of workers in Europe and America attention was directed to the possible occurrence of psittacosis in Australia Prior to 1934 medical literature contained no references to the possible occurrence of human psittacosis in that country However in this same year Meyer and Eddie (1934) succeeded in isolating the virus from a consignment of 200 Australian parakeets which had been imported into California from Sydney Thereafter Burnet and Macnamara (1936) together with other Australian workers recovered the virus from a series of human cases and formally established the presence of the malady in Australia

Public health legislation banning the importation of parrots into Great Britain was introduced in April 1930 (Special Regulations

FRANCIS B GORDON, M D

2 The Psittacosis Viruses A General Survey

I HOPE to contribute to the background of this symposium by some general remarks on the ecology of the psittacosis agents to be followed by a somewhat more detailed review of studies with which I have had contact through former students or other colleagues

Ecology

I wish first to call your attention to the wide and diverse host range of the psittacosis viruses In 1942 Meyer (*1*) listed 31 species of birds in the family Psittacidae and 15 species in five other fami lies of non psittacines which had been found naturally infected in the open or in aviaries The list of non psittacine species found in fected in nature has increased to 20 or more at this date (*2*) and both psittacine and non psittacine birds infected with these viruses have been found in many parts of the earth

In considering the viruses of this group whose natural hosts are found among the mammals at least one that of lymphogranuloma venereum (LGV) is uniquely associated with man The same is true of the viruses of trachoma and inclusion blenorrhea although these are not considered to be full fledged members of the group under discussion Whether man is a natural host of other viruses of this group is questionable The San Francisco (SF) (*3*) Louisi ana (*4*) and Illinois viruses (*5*) sometimes grouped together as the human pneumonitis strains were recovered from man under conditions in which there was no apparent contact with infected

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ing in frequent mutation in this group No direct observations have been reported in support of this postulation Nevertheless the wide diversity of hosts and probable high adaptability of these viruses are of importance in studies concerned with the host range means of transmission and methods of control of these infections

The success of these viruses as parasites is further attested by their high incidence in the various normal host species their tendency to produce mild or nonfatal illness and their tendency to persist for long periods in convalescent or in apparently normal but *infected hosts thus insuring a large number of carriers* When in such a situation the balance between host and parasite is altered in favor of the parasite the infection changes from one which is mild or inapparent to one producing frank disease Among caged birds insanitary conditions such as crowding chilling malnutrition and intensive breeding have been observed to initiate outbreaks Perhaps other factors are responsible for epizootics of psittacosis as observed in wild birds

These host parasite relations have been studied to the greatest extent in the avian viruses either in the natural or in experimental hosts but the generalization can be extended at least to some mammalian strains The tendency for long carriage of virus is strikingly illustrated by a recent report (*17*) of human carriage of psittacosis virus for eight years although no evidence was obtained that the individual concerned was a source of infection

At present we do not have a complete answer to the question of how these viruses are transmitted from host to host in nature Their morphologic resemblance to the rickettsias noted by the early observers raised the possibility of an arthropod vector As the broad ecologic picture of this group has taken shape I presume the question has come to many minds as to whether our present concepts of the natural mode of transmission of these agents can entirely account for the observed facts However I am aware of no evidence at present that bloodsucking arthropods are involved in the maintenance of these viruses

Epidemiologic observations of psittacosis infection provide evidence *that man is highly susceptible to air borne virus presumably* by inhalation This conclusion has been confirmed by a reconstruction and analysis of an accidental laboratory infection (*18*)

animals Nevertheless the conclusion cannot be made that these strains maintain themselves in man alone in spite of some degree of man to man transmission The hypothesis that the Louisiana virus is not strictly 'human is strengthened by a recent report (*6*) of the isolation from snowy egrets of a virus resembling the original Louisiana virus

Viruses morphologically and immunologically related to the avian strains have been isolated from rodents (mouse *7a* and possibly hamster *8*) marsupials (opossums *9*) ungulates (bovines *10a* and sheep *11*), and carnivores (cat *12* and possibly the ferret *13*) In recent studies (*14*) on salmon poisoning in dogs and foxes the possibility has been raised that a virus of this group plays an etiologic role by means of transmission through flukes Although not observed in the fluke morphologic structures with some resemblance to psittacosis virus were observed in the mammals If such an agent properly belongs to the psittacosis group * and occurs in flukes it will be the first instance of a virus of this group being found with certainty in a nonvertebrate animal It is noteworthy however that Thompson and Huff (*15*) in a study of saurian malaria observed in lizard tissue structures strikingly similar to the elementary body containing vesicles of the psittacosis *viruses*

Setting aside the taxonomic problems involved in a precise description of the relationships of these viruses it is obvious that we are dealing with a large group of infectious agents which have successfully parasitized a wide variety of vertebrate hosts in many different geographic locations

Diversity is seen too in the types of transmission that occur These include inhalation of air borne virus (considered to be the usual method) sexual contact in the case of LGV and possibly ingestion in some cases This diversity bespeaks a group of microorganisms or a single prototype which in the evolutionary sense has demonstrated a high degree of adaptability The question has previously been raised (*16*) whether this diversity and apparent adaptability may be due to a degree of genetic instability result

* Since this paper was presented evidence has appeared for the inclusion of this agent in Rickettsiaceae. Philip Hadlow and Hughes *Riassunti delle Communicazioni VI Congresso Internazionale di Microbiologia* 2 256–57 (1953)

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Epidemiologic observations of psittacosis infection provide evidence that man is highly susceptible to air borne virus presumably by inhalation This conclusion has been confirmed by a reconstruction and analysis of an accidental laboratory infection (*18*)

A similar means of transmission probably obtains for both wild and domestic birds Several species have been found infected at an early age and it is supposed that infection of nestlings is the rule (*19*) Inhalation of particles from infected droppings or other discharges is considered the most likely means of transmission although ingestion of contaminated food has also been considered Experimental observations with the mouse pneumonitis virus (*7b*) and with the calf virus (*10a*) indicate that infection can be accomplished by ingestion a means of transmission which probably can occur naturally as well

The possibility of congenital transmission in birds by infected eggs has also been considered The report of Davis and Vogel (*20*) that chicks hatched from eggs infected with psittacosis virus will harbor the virus for 22 days at least and the previous demonstration by Meyer (*1*) of virus in the ovaries and in one case in an egg in the oviduct of infected parakeets form the basis for this postulation

Morphology and Development

The assignment of these several viruses of both avian and mammalian origin to a single group is primarily based on morphologic and immunologic similarities The morphology of these viruses including their developmental cycle has long been one of their most interesting characters and has been studied by many investigators Although minor differences are seen between the members of the group and the interpretations of different investigators are not in entire agreement the essential facts are well established With minimal reference to the differences I shall attempt to present the essential features concerning the morphology and developmental cycle of these viruses For the figures presented here I am indebted to Dr Emilio Weiss

The elementary body established as the smallest recognizable infectious unit of these viruses is readily demonstrated under the ordinary light microscope Figure 1 illustrates a purified preparation of feline pneumonitis virus stained by the Gram method with which it is revealed as Gram positive (*21*)

When elementary bodies of different members of this group are examined by electron microscopy the appearance of the various

members is essentially the same except for minor differences in size (22) The elementary body is composed of a dense inner sub stance circular in outline centrally located with a halo of less dense material (Figure 2) These bodies are probably spherical in the normal hydrated state but shadowed preparations indicate that much distortion and flattening occur during drying The dried particle has more nearly the shape of a low crowned derby hat, or of a fried egg with less than its normal complement of albumin Estimates of the size of the psittacosis virus by filtration through collodion membranes give a value of 200 to 300 mμ and for the size of LGV virus somewhat smaller values (125 to 175 mμ) Studies of this group of viruses by electron microscopy reveal images with diameters of the order of magnitude of 400 to 500 mμ (22) The distortion of the elementary body during drying prob ably accounts for this discrepancy

When elementary bodies are introduced into a susceptible tis sue they develop into particles of larger size the initial bodies (Figure 3) The mechanism of this change is unknown because there is a period of several hours in which no virus elements at all are seen Initial bodies begin to be visible at six hours in the mouse lung infected with mouse pneumonitis at 15 hours with feline pneumonitis and 18 hours with meningopneumonitis virus (23) Bedson and Bland (24) state that small plaques were apparent in mouse spleen infected with psittacosis virus as early as five to six hours after infection Recent reports (25) have suggested a noninfectious phase during growth of the virus of meningopneu monitis during the first 20 hours of growth The correlation be tween these studies and the morphologic observations of others is not readily apparent

Further development of the initial body can take one of several directions depending in part on the virus under observation and in part on the tissue in which the growth is occurring As illus trated in Figure 4 the initial body may produce a small group of particles each of which is somewhat smaller than the initial body Weiss (23) has introduced the term "cluster to describe this par ticular stage in the cycle The previous term "morula" used by Bedson and Bland (24) seems to have been applied to a later stage This particular line of development from the initial body

is exemplified by the growth of the mouse pneumonitis virus in alveoli of the mouse lung The particles of such a cluster increase in number presumably by division and decrease in size progressively until the typical large vesicle is produced containing many elementary bodies often one or more vacuoles and perhaps a plaque Rupture of the mature vesicle releases elementary bodies to repeat the cycle On the other hand if we are observing the meningopneumonitis virus in the alveolus of the mouse lung we see the development from the initial body of a small group of

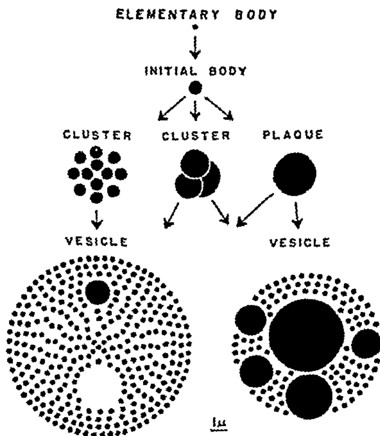


FIGURE 4 Stages in the development of viruses of the psittacosis-lymphogranuloma venereum group Reprinted from Weiss *Journal of Infectious Diseases* 84 125–49 (1949) by permission.

larger bodies termed plaques or perhaps a single plaque This type of development occurs also with psittacosis and lymphogranuloma virus Such clusters and plaques can develop either into the large vesicle just described or into a vesicle containing larger numbers of plaques and large granules The latter type of vesicle is seen when the mouse pneumonitis virus invades the bronchial epithelium of the mouse lung rather than the alveolus and is also seen with LGV virus in the yolk sac as described by Rake and Jones (26) Elementary bodies are formed later from plaques inside the vesicle

The difference between a cluster and a plaque is probably more apparent than real As has been indicated the mouse pneumonitis virus produces clusters in the alveoli in what appears to be an extracellular location and plaques in the bronchiolar epithelium (Figure 5) The same structure may appear to be a cluster with one staining technique and a plaque with another Bedson and Bland (27) were able to demonstrate small bodies within plaques of the psittacosis virus when the preparation was strongly decolorized with acetone The forms of the virus of mouse pneumonitis in the bronchiolar epithelium appear as plaques when stained by hematoxylin eosin azure but when fixed with Zenker acetic acid and stained by Giemsa they appeared as clusters of smaller elements

All investigators who have studied the morphology of these viruses have wrestled with the problem of the nature of the matrix material in which the granular bodies occur One of the earliest views still supported is that the inclusion body is of a dual nature consisting of elements derived both from the virus and from the host cell The contrary view (28) that the inclusion is composed entirely of viral material has also gained support In Weiss s observations on mouse pneumonitis virus the first view appeared to be favored since the extracellular form seen in the alveoli appeared as clusters of small particles or as small finely granulated vesicles As already indicated the same virus in an unquestionably intracellular position (bronchiolar epithelium) gave rise to plaques suggesting that the cell produced the material within the inclusion body responsible for its appearance as a plaque However when the same investigator extended his work to meningopneumonitis

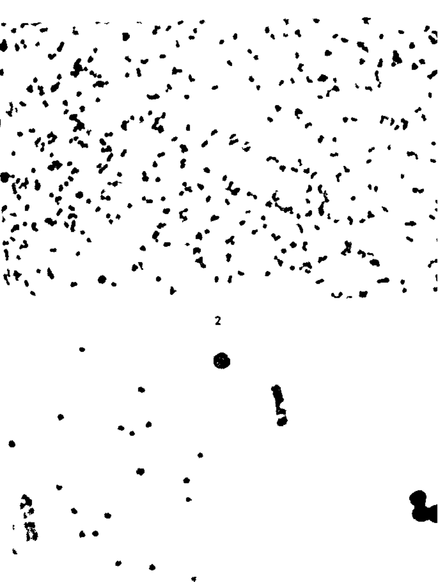
virus he found that plaques rather than clusters developed in what appeared to be an extracellular position in the alveolus of the lung

Minor differences are seen also in the large vesicles of one virus as compared with another The vesicle of mouse pneumonitis virus is fairly rigid maintaining nearly a spherical form Whether observed in the lumen of the alveolus or in the entodermal cells of the yolk sac it deforms the nuclei of cells against which it presses In contrast the feline pneumonitis virus has a less rigid vesicle does not deform nuclei and adapts itself to a greater extent to the contours of its location The mature vesicle reaches a stage in which the matrix material is quite fluid allowing the elementary bodies to be observed in Brownian movement

With mouse pneumonitis virus the entire developmental cycle requires from 30 to 36 hours With the feline pneumonitis virus the time required is 48 hours the figure Bedson and Bland (*24*) have given for the cycle of psittacosis virus

Antigenic Properties

Let us now consider other differences between the viruses of this group and more specifically the practical problem of distinguishing between them or identifying a new member The animal of origin if known and pathogenicity tests on both birds and mammals with particular reference to tissue tropisms (*29a*) aid in differentiation but do not give precise information Immunologic differences can be demonstrated by cross immunization tests in experimental animals (*29b*) but sharp differences are not always seen and there is some lack of agreement on relationships determined by this method The complement fixation test as ordinarily used is specific only for the entire group of viruses Attempts to discover a serologic test of greater specificity led to the use of antiserums prepared in roosters for performing neutralization tests in mice (*30*) (Table I) Athough neutralization tests with mammalian serums have in general not been feasible because of the low titer of such serums the rooster antiserums proved to possess satisfactory neutralizing titers By these tests a high degree of specificity was observed Antiserums against feline pnemonitis and LGV reacted with no other viruses Two mouse pneumonitis strains were indistinguishable from each other and were unrelated



[illegible] (1) [illegible] preparation [illegible] Gram stain [illegible] decolorization [illegible] × [illegible]000 (2) and (3) [illegible] preparation [illegible] with *Staphylococcus* [illegible] cells. [illegible] 5 and 20 [illegible] respectively. [illegible] appear [illegible] after 5 seconds and [illegible] after 20 seconds [illegible] ×4000. [illegible] *Journal of* [illegible] 88 56–67 (19[illegible]) by permission.

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A
B
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C

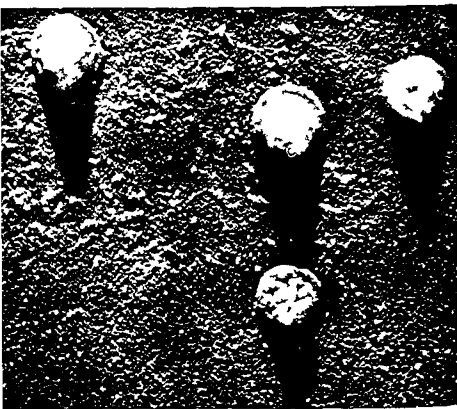


FIGURE 2 Electron micrograph of the agent of feline pneumonitis. Preparation identical with that shown in Figure 1. ×39,000. Reprinted from Moulder and Weiss, *Journal of Infectious Diseases* 88 56–67 (1951), by permission.

FIGURE 3 Developmental stages of the agent of murine pneumonitis. Chick embryo yolk sac tissue sections stained with hematoxylin-eosin azure II. ×[illegible]000. (*a*) Initial body (I) and clusters (C). (*b*) Medium sized vesicle. (*c*) Large vesicles. Reprinted from Weiss, *Journal of Infectious Diseases* 87 249–63 (1950), by permission.


A
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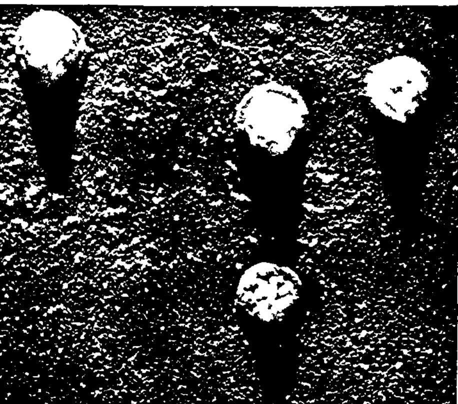


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FIGURE 3 D velopm ntal stages of the agent of murine pneumonitis Chick embryo yolk ac ti sue sections stained with hematoxyl n-eosin azure II ×2000 (*a*) Initial body (I) and clusters (C) (*b*) Medium sized vesicle (*c*) Large vesicles Reprinted from Weiss *Journal of Infectious D seases* 87 249–63 (1950) by permis si n

to any other of the viruses tested, except for partial cross reactions with another similar virus of uncertain origin The meningopneumonitis virus and an ornithosis strain appeared to be identical, a conclusion indicated by other types of tests as well None of

TABLE I Cross neutralization tests with antisera prepared in roosters

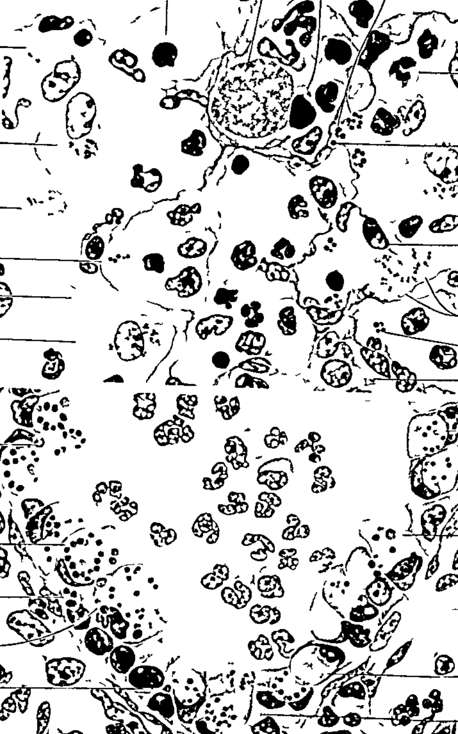
VIRUSES	Antisera					
	Ly venereum	Feline pneum	Meningo pneum	Mouse pneum	Ann Arbor #1	Ann Arbor #2
Ly venereum	+	O	O	O	O	
Feline pneum	O	+	O	O		O
Meningopneum	O	O	+	O	O	+
Ornithosis			+	O		
Mouse pn. (Chicago)	O	O	O	+	±	±
Mouse pn (Nigg)			O	+		
Ann Arbor	O	O	O	+	+	+
Human pn. (SF)	O	O	O	O		
Psittacosis	O	O	O	O		
Illinois	O	O	O	O		

these antiserums mentioned had any effect on a psittacosis strain, the human pneumonitis (SF) or the Illinois virus

Another method of demonstrating a degree of immunologic specificity in the viruses of this group is the toxin antitoxin test developed by Rake and his colleagues (31) and used on a broad scale by Manire and Meyer (32) Insofar as they can be compared there is substantial agreement between the analyses of the group as determined by the serum neutralization and the toxin antitoxin test

The immunologic similarity of these viruses as demonstrated by the complement fixation test is dependent upon an antigen common to the group Two components in complement fixing an

Figure 5 Mouse pneumonitis virus in mouse lung Hematoxylin-eosin azure II C—cluster E—endothelial cell H—heterophile I—initial body L—lymphocyte M—macrophage (labeled with trypan blue) MI multiple infection of single cell P—plaque Vg—finely granular vesicle Pl—polyblast Rbc—red blood cell S—septal cell Vl—large vesicle (1) Extracellular virus in the alveoli (2) Intracellular virus in the epithelium of a bronchiole Reprinted from Weiss *Journal of Infectious Diseases* 84 125–49 (1949) by permission.



(*21*) Preparations of a fraction containing up to 0 3 mg nitrogen and $10^{7\ 8}$ LD_{50} per ml failed to show any oxygen uptake with glucose pyruvate glutamate alpha ketoglutarate and succinate as substrates No lactate formation from glucose was detected and glucose was not phosphorylated in the presence of adenosine tri phosphate In contrast typhus rickettsias under similar conditions will oxidize pyruvate and glutamate indicating a capacity for in dependent metabolism on the part of the latter microorganisms (*38*) The respiration of the extra embryonic membranes of normal chick embryos and chick embryos infected with feline pneumonitis embryos was studied (*39*) Both living and dead embryos were used since the virus of feline pneumonitis will multiply in the lat ter if the tissues are still metabolizing (*40*) No differences were observed in oxygen uptake and anaerobic glycolysis between in fected and noninfected tissues Multiplication of the virus was seen to be closely related to the level of metabolism of the infected membranes

In a similar type of experiment (*41*) with mouse lung tissue taken from mice previously infected with mouse pneumonitis or feline pneumonitis virus a very different result was obtained In this case there was a decrease in oxygen uptake and an increase in anaerobic glycolysis compared with normal lung These changes in metabolism although proportional to the degree of consolida tion in the lung were not attributed to the action of the virus Mouse lungs in which a pneumonia had been induced by intranasal instillation of normal egg yolk gave similar results The observed metabolic changes were therefore attributed to the inflammatory response rather than to the virus itself

Conclusions

The psittacosis viruses for various reasons have been considered to be closely related to the rickettsias and with them intermediate between the smaller viruses and the cultivable bacteria In con cluding this survey I should like to point out that these biochemi cal studies reveal that members of the psittacosis group have much stronger affinities to the smaller viruses than to the rickettsias The indications for the psittacosis viruses are a complete dependence

tigen were early shown by Bedson (33) to be distinguishable by their varying resistance to heat Other observations (34) indicate that the heat stable antigen is carbohydrate in character and is common to the entire group of viruses, while the heat labile com ponent probably a protein is of a more specific character Or dinarily the specific antigen is masked by the presence of the group antigen but Bedson and his colleagues (35) have shown that absorption of psittacosis or LGV antiserum by the heat stable common antigen of the group imparts a specificity for the homologous virus to the absorbed serum when employed in the comple ment fixation test Attempts by this group of investigators to provide strain specific antigens were also promising Treatment of the complete antigen with potassium periodate was found to destroy the group factor and provide a specific antigen for use in the complement fixation test Extraction with acid accomplished the same purpose when the preparation was used as a skin test anti gen

A group specific lipid antigen demonstrable by complement fixation has been described by Hilleman and Nigg (36)

Other serologic tests whose usefulness has recently been inves tigated for studies or diagnosis within this group of viruses are the indirect complement fixation the conglutinating complement absorption and the hemagglutination inhibition tests (37) In every case group specificity was encountered The identity or lack of identity of many of these various immunologically active components is unknown

Biochemical Investigations

The viruses of this group being of large size and cultivable in high concentrations have not been particularly difficult to pre pare in relatively purified form It is surprising therefore that these viruses were not exploited earlier for chemical studies Re cently Moulder and his colleagues have reported chemical studies which I will mention briefly

After purification of the virus of feline pnemonitis to a rela tively high degree tests were performed to detect respiratory activity of this preparation in the presence of various substrates

28 Cf Rake *J Bacteriol* 54 637–40 (1947)
29a Beck, Eaton and O Donnell *J Exper Med* 79 65–77 (1944)
b Cf Wagner Golub and Andrew *J Infect Dis* 84 41–46 (1949)
30a Hilleman *J Infect Dis* 76 96–114 (1945)
b St John and Gordon *J Infect Dis* 80 297–306 (1947)
31a Rake and Jones *J Exper Med* 79 463–86 (1944)
b Hamre and Rake *J Infect Dis* 74 206–11 (1944)
32 Manire and Meyer *J Infect Dis* 86 226–32 233–40 241–50 (1950)
33 Bedson *Brit J Exper Pathol* 17 109–21 (1936)
34a Nigg Hilleman and Bowser *J Immunol* 53 259–68 (1946)
b Barwell *Nature* 162 460–61 (1948) 164 1013 (1949)
35 Bedson *et al* *J Clin Pathol* 2 241–49 (1949)
36 Hilleman and Nigg *J Immunol* 53 201–08 (1946) 59 349–64 (1948)
37 Hilleman Haig and Helmhold *J Immunol* 66 115–30 (1951) 68 121–29 (1952)
38a Bovarnick and Snyder *J Exper Med* 89 561–65 (1949)
b Bovarnick and Miller *J Biol Chem* 184 661–76 (1950)
39 Moulder and Weiss *J Infect Dis* 88 68–76 (1951)
40 Weiss *J Infect Dis* 86 27–32 (1950)
41 Moulder and Weiss *J Infect Dis* 88 77–80 (1951)

for growth on actively metabolizing tissue and no evidence of any independent metabolism Both of these observations contrast with known properties of the rickettsias

The evidence of drug susceptibility has also been applied to the question of relationships of the psittacosis group General as well as specific information on the susceptibility of these viruses to drugs is to be covered in a later paper

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16 Gordon *Ann Rev Microbiol* 4 151–68 (1950)
17 Meyer and Eddie *J Infect Dis* 88 109–25 (1951)
18 Rosebury *et al* *J Infect Dis* 80 64–77 (1947)
19a Burnet *J Hyg* 35 412 (1935)
b Cf 1
20 Davis and Vogel *Proc Soc Exper Biol and Med* 70 585 (1949)
21 Moulder and Weiss *J Infect Dis* 88 56–67 (1951)
22 Cf Kurotchkin *et al* *J Immunol* 55 283–87 (1947)
23 Weiss *J Infect Dis* 84 125–49 (1949)
24 Bedson and Bland *Brit J Exper Pathol* 13 461–66 (1932)
25 Sigel *et al.*, *J Exper Med* 94 401–13 (1951)
26 Rake and Jones *J Exper Med* 75 323 (1942)
27 Bedson and Bland *Brit J Exper Pathol* 15 243–47 (1934)

A rapid labored respiration and an associated cough may be observed in about half of the sick animals At the same time diarrhea appears It may be mild or severe the stools are apt to be watery and blue gray in color Within a few days (or at most two weeks after onset) calves have difficulty in walking There is at first stiffness of the gait and knuckling of fetlock joints The animal staggers circles and falls The head is retracted in a position of opisthotonos Finally the limbs are weak with apparent paralysis and soon the affected animal is unable to get up on its feet

Some data on epizootics in 21 herds (4) observed during the course of the study appear in Table I There were 1 774 cattle in these herds Of these 892 were less than one year of age 400 were one to three years old and 482 were adult animals Calves under

TABLE I Summarized morbidity and mortality data on 21 herds affected by sporadic bovine encephalomyelitis

Ages †	*Total*	*Number sick*	*Herd morbidity rates (%)*	*Number dead*	*Herd mortality rates (%)*	*Case mortality rates (%)*
Adults	482	24	5	9	2 0	38
Yearlings	400	19	5	2	0 5	11
Adults and yearlings	882	43	5	11	1 0	26
Calves	892	224	25	64	7 0	29
TOTAL	1774	269	15	75	4 0	28

Data concerning the twenty second herd were incomplete and therefore were omitted

† Calves under 1 year yearlings 1 to 3 years adults 3 years and over

six months of age were uniformly much more susceptible to the disease than older animals Morbidity rates among older cattle averaged 5 per cent whereas among calves the rate was 25 per cent Although calves experienced the greatest herd mortality the observed case mortality was only 29 per cent while adult animals when they did develop the disease had a case mortality of 38 per cent Yearlings had a case mortality of 11 per cent

Autopsies were made on 16 animals All except two were calves brought during illness to the Veterinary Clinics at the South Dakota State College or at the University of Missouri The gross pathologic changes are so well defined that a diagnosis can be

HERBERT A WENNER, M D

3

Sporadic Bovine Encephalomyelitis [1, 2]

From various parts of the world in recent years there have come descriptions of diseases in animals associated with agents belonging to the psittacosis lymphogranuloma group Available information on infection in birds with agents of this group became a bedrock for the scaffold of new information on similar diseases in wild and domestic mammals Comparatively speaking information on the subject of diseases in mammals caused by members of the psittacosis group is much more meager than on diseases in birds A number of new pertinent studies have been made particularly in regard to infection in mice cats opossums sheep and calves

In the midwestern areas of the United States there occurs a disease of cattle (*1 2 3*) known as sporadic bovine encephalomyelitis (BE) characterized by a febrile illness and subsequently in some opisthotonos and paralysis of the limbs During the acute phase of illness there is anorexia depression and inactivity A serous fluid drains from the nostrils There is increased salivation and drooling

[1] Aided in part by a grant from the National Foundation for Infantile Paralysis and the Communicable Disease Center Department of Health, Education and Welfare United States Public Health Service

[2] Acknowledgment is made to the following who collaborated with me or gave assistance during the studies Robert W Menges and M L Furcolow Communicable Disease Center Kansas City Field Station Kansas City Kansas Gerald S Harshfield South Dakota State College, Brookings, South Dakota Loren D Kintner Veterinary Clinic, University of Missouri Columbia, Missouri J L. Melnick, Yale University New Haven Connecticut, David B Lackman and Robert K Gerloff National Microbiological Institute Rocky Mountain Laboratory Hamilton Montana.

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(a)



(b)



FIGURE 1. Microscopic pathological [illegible] tissue obtained from the [illegible] cerebellum of [illegible] cephalomyelitis. (a) Organized [illegible] exudate showing vascular proliferation and organization of thrombi in capillaries. (b) Infiltration of parenchymal tissue of cerebellum with round cells. [illegible] presumably ischemic. Reprinted from [illegible] Harshfield [illegible] Wenner, *American Journal of Hygiene* [illegible] (1953) by permission.

FIGURE 2. Electron microphotograph of [illegible] experimentally infected yolk sac. Approximately [illegible] ×18,400. Reprinted from Wenner, Harshfield, Chang, and Menges, *American Journal of Hygiene* [illegible] (19[illegible]) by permission.

made at post mortem with reasonable certainty Always there has been peritonitis serofibrinous in nature Early in illness there may be free, yellow watery fluid in the peritoneal cavity Later, at a time when encephalomyelitis is clinically apparent the exu date thickens and forms a fibrinous net enveloping the omentum and covering the surfaces of the liver and spleen Delicate ad hesive strands of fibrin bind the omentum to the spleen and liver and the liver to the diaphragm The spleen may or may not be enlarged A fibrinous pleuritis and pericarditis similar in appear ance to that observed in the peritoneal cavity is almost invariably found

I shall not report on details of microscopic pathology These have been reported in a previous paper (3) There is fibrinous exudate involving serous membranes mentioned above (Figure 1) The findings in the central nervous system are of greater interest A diffuse inflammatory process occurs involving the brain and spinal cord Meningitis myelitis and encephalitis are found on microscopic examination of affected tissues

The meningeal reaction is characterized by the presence of an organizing exudate with mononuclear cells in the older lesions and by the presence of both mononuclear and polymorphonuclear cells enmeshed in a fibrinous exudate in the more recently acquired illnesses In the cord proper intensive inflammatory lesions in volve the gray matter There is extensive vascular damage An associated degeneration and destruction of neurons accompanies the vascular injury Minute granulomatous abscesses are observed in both the gray and white matter of the cord The lesions are sug gestive of secondary changes following generalized vascular dam age and indicate a sweeping injury in which all areas of the cen tral nervous system appear to be involved at the same time

Studies on the Etiology of the Disease

Through the kindness of Dr Kintner there was made available to us central nervous system (CNS) tissues obtained from calves ill with encephalitis In the beginning of the study we failed to appreciate the significance of the serofibrinous exudate and our methods were directed at the isolation of neuronotropic virus When we repeated McNutt s (5) and Harshfield s (2) tests of

infected material in guinea pigs and found that these animals develop fever and a fibrino purulent peritonitis which is bacteria free a search was made for elementary bodies These minute bodies are hard to find in exudates whether obtained from calves experiencing naturally acquired infection or from experimentally infected guinea pigs They have never been numerous and I suspect that they can be missed although a careful search of well prepared slides should reveal them The next step namely that of growing the agent in the yolk sac of the developing hen s egg was readily accomplished In the course of our studies nine strains of an agent infectious for guinea pigs were detected in tissues obtained at post mortem from 12 cattle (6)

Properties of BE Virus

It has been relatively easy to find minute coccal bodies in infected yolk sac impression smears and comparatively difficult to find them in exudate or impression smears of tissues obtained from experimentally infected mammals The coccal bodies vary in size take a red stain with basic fuchsin and are more readily decolorized with citric acid than is the meningopneumonitis virus Electron microphotographs (Figure 2) of elementary bodies gave an average size of about 375 mμ The virus is thermolabile it is inactivated within 15 minutes at 62 C Two strains have remained viable in storage at −70 C for 24 months

Transmissions of the Disease to Calves with BE Virus

Three strains of BE virus were inoculated into calves in order to demonstrate an etiologic relationship of the virus to the natural disease observed in cattle (7) With each strain a similar clinical syndrome was found There was fever depression anorexia and weight loss Excepting mild disturbances in gait none of the experimental calves developed clinical evidence of encephalitis Each of the experimental calves was found to have visceral lesions which we consider pathognomonic of the disease namely peritonitis pleurisy and pericarditis Four of the 16 calves had histologic evidence of meningitis and encephalitis but not of a degree corresponding to the CNS changes observed in the naturally occurring

ALF Exp 114	Dec 5	1	19	26	Jan 1		12	19	6	Feb 1
						↓ BEV W IP				Sacrificed 2/3/53

Temp F: 105, 104, 103, 102, 101

Serology	Dec 5	Jan	Feb
Lansing Polio	<1 2	<1 2	<1 2
BEV	1 4	1 4	1 256
LGV	1 16	1 16	1 64
Psittacosis	1 4	1 4	1 768
Q Fever	1 4	<1 4	1 16
RMSF	1 4	1 24	<1 4

Virus Isolation	
STOOL	0/3 G P
BLOOD	+ G P
NASAL DISCHARGE	0/3 G P

HISTORY OF STRAIN

W Strain Isolated in 1952 from CNS obtained from a calf Passaged successfully in guinea pigs lethal for chick embryos When used the strain was in the third passage in embryonated eggs

FIGURE 3 Clinical course of experimental Calf 114 inoculated intraperitoneally with a strain of bovine encephalomyelitis virus Reprinted from Wenner Meng s and Harshfield *Journal of Infectious Diseases* 94 84 (1954)

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It has been relatively easy to find minute coccal bodies in infected yolk sac impression smears and comparatively difficult to find them in exudate or impression smears of tissues obtained from experimentally infected mammals The coccal bodies vary in size take a red stain with basic fuchsin and are more readily decolorized with citric acid than is the meningopneumonitis virus Electron microphotographs (Figure 2) of elementary bodies gave an average size of about 375 mμ The virus is thermolabile it is inactivated within 15 minutes at 62 C Two strains have remained viable in storage at —70 C for 24 months

Transmissions of the Disease to Calves with BE Virus

Three strains of BE virus were inoculated into calves in order to demonstrate an etiologic relationship of the virus to the natural disease observed in cattle (7) With each strain a similar clinical syndrome was found There was fever depression anorexia and weight loss Excepting mild disturbances in gait none of the experimental calves developed clinical evidence of encephalitis Each of the experimental calves was found to have visceral lesions which we consider pathognomonic of the disease namely peritonitis pleurisy and pericarditis Four of the 16 calves had histologic evidence of meningitis and encephalitis but not of a degree corresponding to the CNS changes observed in the naturally occurring

syndrome Each of the experimental calves inoculated with BE virus developed during the course of illness specific serum complement fixing antibodies against BE virus A diagrammatic summary of the clinical course and of some studies made on one of the experimental calves appears in Figure 3

Serologic Relationships of BE Virus to the Psittacosis-LGV Group

Guinea pigs were immunized with strains of BE psittacosis, and ornithosis viruses Using antigens prepared from the same viruses there was a strong group relationship The results of one of these serologic tests (*8*) appear in Table II Serums obtained from calves inoculated with BE virus reacted in complement fixa

TABLE II Studies on serologic relationships of bovine encephalomyelitis virus and members of the psittacosis lymphogranuloma venereum and rickettsial groups

(DIRECT COMPLEMENT FIXATION)

ANTISERUM PREPARED IN GUINEA PIGS	Antigens					
	Psittacosis	*BEV*	*RMSF*	*Q fever*	*Ornithosis*	*Saline control*
Psittacosis	256	16	Trace	4	—	0
BEV	512	128	16	0	0	0
RMSF	0	0	512	—	—	0
Q fever	0	4	32	512	0	0
Boutonneuse fever	8	0	128	—	—	0
Ornithosis	—	0	—	0	32	0
Normal guinea pig serum	0	0	0	0	0	0
Saline	0	0	0	0	0	0

BEV—bovine encephalomyelitis virus RMSF—Rocky Mountain spotted fever
From data kindly supplied to me by D B Lackman and R. K. Gerloff

tion test with BE lymphogranuloma venereum (LGV) psittacosis and ornithosis antigens serum titers were high in all instances so that had the agent been unknown a diagnosis would have been impossible The virus and its specific antiserum did not react with Q fever or endemic typhus antiserums or antigens

The Pathogenesis of BE

Our observations on the natural disease in cattle and our experimental studies in calves indicate that encephalitis is incidental to a systemic infection in which every organ system is potentially if not actually at risk of attack by the infectious agent Evidence in support of a widespread distribution of the virus in body tissues is based on pathologic lesions which we have observed in affected calves Of nine strains isolated three have been found in pooled emulsions of liver and spleen obtained from calves experiencing sporadic bovine encephalomyelitis the remaining have been found in emulsions of brain stem cerebellum and cervical spinal cord

During our experimental studies in calves samples of blood feces and nasal discharges were collected for virus isolation Although these studies are incomplete at this time BE virus (J W strain) has been recovered from blood (Figure 3) obtained during the acute phase of illness in three calves Four attempts to isolate virus from nasal discharges yielded negative results nor has the virus been recovered from feces Contrary to Baker s experience (9) inoculation of feces obtained from the experimental calves has not produced fibrinous peritonitis in guinea pigs

The Epizootiology of the Disease

The greater susceptibility of calves less than six months of age as compared with adult cattle suggested that subclinical infection probably occurred frequently resulting in a relative or absolute immunity in many adult cattle Older cattle seldom contract the disease in the form described for calves but when they do the case mortality rate is as high as or may be higher than those observed in calves (3 4)

The disease is endemic over a wide area of the Middle West (Figure 4) Epizootics occur in herds containing large numbers of calves and not infrequently outbreaks follow recruiting of susceptible calves into established herds Under these conditions the velocity of infection is apt to be rapid and the duration of herd illness to span a period of about four weeks

Serologic studies of members of affected herds indicate a limited dispersal of virus within it Not only do some animals not develop

illness but some animals do not develop complement fixing anti bodies one or two months after onset of the epizootic An estimate at this time indicates that roughly 50 per cent of animals become infected the majority experiencing inapparent infections Ob-

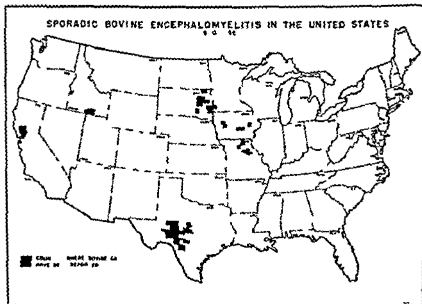


Figure 4 Sporadic bovine encephalomyelitis in the United States 1940-1952 Reprinted from Menges Harshfield and Wenner *Journal of the American Veterinary Medical Association* 1_2 294 (1953) by permission

servations on herd outbreaks have suggested to us that infection is acquired by the oral or respiratory portals The failure to find BE virus in nasal exudate or in feces during the acute phase of ill ness in calves does not augur well for this postulate The presence of virus in the circulating blood suggests that it may find its way into milk or that a biting arthropod vector is responsible for trans mission of the agent to susceptible calves and older cattle

The Relationship of BE Virus to Other Viruses of the Same Group, with Particular Reference to Mammalian Infections

Having arrived in the pursuit of an agent belonging to the psittacosis LGV group by chance and actually quite uninitiated I soon found that to categorize BE virus as a separate and distinct virus from others of the group was indeed a very difficult and frustrating effort We have not made a comparative study of BE virus and its relationship to other agents isolated from mammalian sources What I have done is to survey a large literature and correspond with a number of people in order to obtain some comparative data Those who have done this before me might be sympathetic for from unpublished data I am sure that it is possible to fill in empty spaces appearing in the tables and thereby make the tabular data much more complete although hardly less certain on antigenic differences which exist among members of the group

One of the difficulties is the relative lack of differentiating features by which to distinguish the individual viruses Pathogenicity tests are not very helpful except in psittacosis for which the intraperitoneal inoculation of mice may be helpful I have summarized pathogenicity of mammalian members of the group in Table III Much desirable information is missing Information tabulated certainly indicates host susceptibility but the differentiation of members by this means cannot be readily made Differences in pathogenicity of various agents of this group by pathogenicity tests in birds will appear elsewhere in this symposium

The use of potent type specific neutralizing antiserums has been helpful in delineating antigenic differences Unfortunately hardly any published data are available excepting on psittacosis meningopneumonitis and ornithosis viruses Serum neutralization tests have revealed no relationship among meningopneumonitis human pneumonitis (SF strain) psittacosis (6 BC strain) mouse pneumonitis feline pneumonitis and lymphogranuloma venereum viruses

Toxin neutralization tests have been quite specific in delineating group relationships among these agents (Table IV) Unfortu

TABLE III Peculiarities in host range of the psittacosis lymphogranuloma venereum group *Summary of pathogenicity tests for certain mammals*

Agent	CE Yolk Sac	*Mice*			*Guinea Pigs*			*Monkeys*			*Cats*			*Hamsters*		*Rabbits*		*Original Host*
		IN	IP	IC	IN	IP	IC	IN	IP	IC	IN	IP	IC	IN	IC	IN	IP	
Baker's calf	+	+*	0			+					0							Silent +
BEV	+	0	0	0	ND	+	+	ND	+	+	ND	ND	ND					+
MP	+	+	+/c‡	+	fever ±	fever ±	fever ±		+	+				+	+			
Opossum—A	+	+	0	+/c		0	0			0								+
Sheep pneumonia	+	+									0							+
Abortion (Ewes)	+																	+
Feline pneumonitis	+	+	silent 0/+	silent 0/+	+						+	fever ±		+		+		+
Mouse pneumonitis	+	+†	0	0/c		0								+			0	+
LCV	+	+	0	+/c		0			+	+				±	±			+
Psittacosis	+	+	+	+/c		fever 0	±	+/t		+				+	±	+		+
Ornithosis	+	+	±/c	+										+				+

CE—Chick embryo IN—intranasal IP—intraperitoneal IC—intracerebral
c—carrier stage +/t—intrabacterial ND—not done
After 20 successive transfers
† After 5 or 6 passages
‡ Originally reported negative refers here to MP Cal 10

TABLE IV Summary of some immunologic relationships among indicated members of the psittacosis lymphogranuloma venereum group

Ag	T n Neutralization Tests												Complement Fix tion T sts (d t)												C s Imm unt on											
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12
1 Mouse pneumonit (F cl)	+	0	0							0			+									P/0			+						+			P/0	P	P
2. Fel ne pne monitis	0	+	0							0				+											0	+								0		
3 M use pneumon ti (N gz)	P	0	+							0			+		+							+			P									P		
4 Mouse pneumon t s (De Burgh)				+												+																				
5 Abort on, Ewe					+												+						+													
6. Sheep pneumonitis						+								+				+							+											
7 Oposs m							+												+																	
8. Calf (feces)								+					ND I w ND							+	no	+	ND	ND												
9 BEV									?				?								+															
10 LGV	0	0	0							+	0	0	+									+	+	+	P		P						P	+		
11 Psittacosis										0	+											+	+	+	P									P/0		P
12. Ornithosi											0	+									+	+	+	+	P										+	+

?—wi h roost serum
P/0—30% p otection or >
P—75% protection r >
ND—not done

TABLE III Peculiarities in host range of the psittacosis lymphogranuloma venereum group Summary of pathogenicity tests for certain mammals

Agent	CE Yolk Sac	*Mice*			*Guinea Pigs*			*Monkeys*			*Cats*			*Hamsters*		*Rabbits*		*Original Host*
		IN	IP	IC	IN	IP	IC	IN	IP	IC	IN	IP	IC	IN	IC	IN	IP	
Baker's calf	+	+	0			+					0							Silent
BEV	+	0	0	0	ND	+	+	ND	+	+	ND	ND	ND					++
MP	+	+	+/c‡	+	fever ±	fever ±	fever ±		+	+								
Opossum—A	+	+	0	+/c		0	0			0				+	+			
Sheep pneumonia	+	+																+
Abortion (Ewes)	+										0							+
Feline pneumonitis	+	+	silent 0/+	silent 0/+	+							fever ±						+
Mouse pneumonitis	+	+‡	0	0/c		0					+			+		+		+
LGV	+	+	0	+/c		0			+	+				+			0	+
Psittacosis	+	+	+	+/c		fever								±	±			+
Ornithosis	+	+	±/c	+		0	±	+/t		+				++	±	+		++

CE—Chick embryo IN—intranasal IP—intraperitoneal IC—intracerebral
c—carrier stage +/t—intrabacterial ND—not done
* After 20 successive transfers
† After 5 or 6 passages
‡ Originally reported negative refers here to MP Cal 10

TABLE IV Summary of some immunologic relationships among indicated members of the psittacosis lymphogranuloma venereum group

Agent	Toxin Neutralization Tests												Complement Fixation Tests												Cross Immunization											
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12
1 Mouse pneumonitis (Francis)	+	0	0							0			+									P 0			+						+			P/0	P	P
2. Feline pneumonitis	0	+	0							0				+											0	+								0		
3 Mouse pneumonitis (Nigg)	P	0	+							0			+		+							+			P									P		
4 Mouse pneumonitis (De Burgh)				+												+																				
5. Abortion, Ewe					+												+						+													
6. Sheep pneumonitis						+								+				+							+											
7 Opossum							+												+																	
8. Calf (feces)								+					ND	low	ND					+	lo	+	ND	ND												
9 BEV									?				?								+															
10 LGV	0	0	0							+	0	0	+									+	+	+	P		P						P	+		
11 Psittacosis										0	+											+	+	+	P									P 0		P
12. Ornithosis											0	+									+	+	+	+	P										+	+

?—wi h conter se um
P/0—30% protection or >
P—75% protection or >
ND—not done

TABLE III Peculiarities in host range of the psittacosis lymphogranuloma venereum group Summary of pathogenicity tests for certain mammals

Agent	CE Yolk Sac	*Mice*			*Guinea Pigs*			*Monkeys*			*Cats*			*Hamsters*		*Rabbits*		*Original Host*
		IN	IP	IC	IN	IP	IC	IN	IP	IC	IN	IP	IC	IN	IC	IN	IP	
Baker's calf	+	+	0			+					0							Silent +
BEV	+	0	0	0	ND	+	+	ND	+	+	ND	ND	ND					+
MP	+	+	+/c‡	+	fever ±	fever ±	fever ±		+	+				+	+			
Opossum—A	+	+	0	+/c		0	0			0								+
Sheep pneumonia	+	+									0							+
Abortion (Ewes)	+																	+
Feline pneumonitis	+	+	silent 0/+	silent 0/+	+						+	fever ±		+		+		+
Mouse pneumonitis	+	+†	0	0/c		0								+			0	+
LGV	+	+	0	+/c		0			+	+				±	±			+
Psittacosis	+	+	+	+/c		fever 0	±	+/t		+				+	±	+		+
Ornithosis	+	+	±/c	+										+				+

CE—Chick embryo IN—intranasal IP—intraperitoneal IC—intracerebral
c—carrier stage +/t—intrabacterial ND—not done
After 20 successive transfers
† After 5 or 6 passages
‡ Originally reported negative refers here to MP Cal 10

TABLE IV Summary of some immunologic relationships among indicated members of the psittacosis lymphogranuloma venereum group

Agent	Toxin Neutralization Tests												Complement Fixation Tests (direct)												Cross Immunization											
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12
1 Mouse pneumonitis (Francis)	+	0	0							0			+									P/0			+						+			P/0	P	P
2. Feline pneumonitis	0	+	0							0				+											0	+								0		
3 Mouse pneumonitis (Nigg)	P	0	+							0			+		+							+			P									P		
4 Mouse pneumonitis (De Burgh)				+												+																				
5 Abortion Ewe					+												+						+													
6. Sheep pneumonitis						+								+				+							+											
7 Opossum							+												+																	
8. Calf (feces)								+					ND	low	ND					+	o	+	ND	ND												
9 BEV									?				?								+															
10 LGV	0	0	0							+	0	0	+									+	+	+	P		P						P	+		
11 Psittacosis										0	+											+	+	+	P									P/0		P
12. Ornithosis											0	+									+	+	+	+	P										+	+

?—with rooster serum
P/0—30% protection or >
P—75% protection or >
ND—not done

nately, with regard to viruses under discussion there is an area barren of information in a field worthy of exploration Dr Karl Meyer has informed me that the WS strain of BE virus is a poor toxin producer We have not had opportunity to study other strains in this regard

The direct complement fixation test has been of little value in distinguishing individual members of the group from one another since they show group specificity rather than strain specificity by this test Indirect complement fixation has been somewhat better in setting apart viruses in the group

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DOUGLAS H SPRUNT, M D

4 The Pathology of Psittacosis

THE pathologist of diseases in man is frequently limited in his study because a given disease may affect only one animal host or at best several mammals In psittacosis however he has the advantage of seeing what the disease agent can do not only in several mammals but also in a number of different species of birds In this way he gets a better understanding of the basic nature of the disease agent

My first contact with psittacosis was in 1930 at which time I had the privilege of working with Drs Thomas M Rivers and George P Berry I have listed in the bibliography only a few of the out standing papers on the morbid anatomy of psittacosis as the litera ture has been well reviewed by Lillie in his papers

Nature of Histologic Changes

Before proceeding to the specific lesions in the various animals I should like to discuss briefly the fundamental nature of psitta cosis Its reaction like that of most viruses rickettsias and some bacteria (particularly those bacteria which are associated with a strong exo or endotoxin in necrotizing) is proliferative in con tradistinction to the exudative type of reaction which is com monly associated with pyogenic infections

Experimentally it is possible to produce with certain viruses either a necrotizing or proliferative reaction by varying either the strength of the virus or the susceptibility of the host A highly virulent virus or a highly susceptible host results in a necrosing

lesion whereas a weaker virus or a more resistant host results in a proliferative lesion

In the bird with psittacosis we have evidences of both prolifera tion and necrosis In the birds which are most resistant and which are carriers of the virus the lesion is primarily proliferative whereas in the animals which are seriously ill with the disease necrosis is generally present The lesion in which there is consider able necrosis is also the one in which the virus itself is the most easily found The virus can be found at times however in the spleen of birds which do not have much necrosis The spleen of the bird does not have the distinct separation of malpighian cor puscles and pulp that is found in mammals but even the slight separation which does exist disappears when the bird is infected with psittacosis and the cells are damaged to some extent, an occasional necrotic cell being seen

In considering the pathology of a disease it is important to consider whether the changes are the result of the presence of the disease agent or are the result of the general toxicity of the disease If the disease agent is found in a tissue it is pre sumed that the lesion is the result of its presence In the bird the disease agent may be most readily found in the spleen the liver and the pericardium It is also at times recovered from the kid neys intestines and lungs but as far as we know never from the brain In mammals the location of the virus is essentially the same except that the lung of mammals contains large amounts of virus the lung of the bird seldom contains appreciable amounts of virus

Liver Lesions in Birds

Although there are no studies on the hematologic changes in birds there is evidence of extensive destruction of red cells This is apparent in the liver and spleen by the presence of large amounts of hemosiderin Some workers have thought that the areas of necrosis in the liver were due to the presence of this iron contain ing pigment however such areas are found when not associated with hemosiderin The liver of the bird also contains areas of eosinophilic necrosis which as a rule are not infiltrated with cells

The parrot develops in addition what might be called an acute

biliary cirrhosis Other birds may also have this lesion but we have seen it only in the parrot s liver and have not found it de scribed by other workers as present in other birds The funda mental nature of this lesion is due to the anatomic structure of the liver and to the more extensive blood destruction in the parrot as compared to other birds Birds have no separate lymph nodes but in various organs have a considerable amount of lymphoid tissue This lymphoid tissue may be separated from the other tissue by connective tissue In the liver in the periportal region there is a focal accumulation of lymphoid tissue around the bile ducts This tissue together with the duct is separated from the rest of the liver by fibrous tissue capsule In the study of a number of livers many by serial section the earliest change seen in psittacosis is a proliferation of this lymphoid tissue This proliferation results in compression of the bile duct This together with the increased bile formation due to the blood destruction causes the ducts to be come plugged The wall of the duct also becomes necrotic and fre quently ruptures When this occurs bile pours out among the lymphocytes which are around the bile duct and within the fibrous capsule The lymphocytes in this area are either replaced or changed to macrophages which contain large amounts of bile pigment This change of type of cell occurs so quickly that it ap pears quite likely that the lymphocytes are changed to macro-phages The bile also infiltrates the surrounding tissues and causes a necrosis of the liver cells There is moreover a proliferation of bile ducts

Other Lesions in Birds

On the pericardium there are a few round cells and the ele mentary bodies of psittacosis are seen In the bird the lung is generally not involved We have however seen lesions in four parrots similar to those that occur in the monkey (described be-low) The virus was isolated from these lung lesions

The kidneys show some parenchymatous change and also an occasional interstitial proliferative lesion has been described This lesion has rarely been observed by us The mucosa of the intestines occasionally shows some hyperplasia

The most prominent lesion in mammals occurs in the lungs The

sputum of man is infectious, and many man to man infections have been reported Our personal experience has been largely with monkeys which were infected by intratracheal injections of the virus and then killed at various stages throughout the disease and during recovery We have also studied sections from several human cases The lesions in man and monkey seem to be essentially the same

Lung Lesions in Man

The lesion in man is almost entirely proliferative and is limited we believe to the lung The infectious agent can be recovered from the lung but can rarely be stained in the lung tissue Since the disease starts in one lobe and spreads successively through all the lobes it is difficult to correlate time with the anatomic changes In one instance the disease lasted over three weeks and all five lobes were involved

The changes in the lung can best be understood by a brief review of the anatomy of the lung For years there has been an argument as to whether the alveoli of the lung were lined with epithelial cells or not We believe that anyone studying the lungs from either man or monkey that died of psittacosis can have little doubt that the alveoli are lined with epithelial cells as the proliferation of these cells is a predominant feature of the lesion In the earliest phase of psittacosis there is a slight polymorphonuclear exudate which disappears unless a secondary pyogenic infection occurs This however is not common in psittacosis as it is in other viral diseases like measles Perhaps this is because there is not much damage to the bronchial epithelium in psittacosis This slight exudate of polymorphonuclear leucocytes is followed within 24 hours by red blood cells fibrin and macrophages The most dominant part of the lesion however is proliferation of the epithelial lining cells Many of these cells have sloughed off into the lumen of the alveoli so that the alveoli are frequently filled with a large number of large mononuclear cells some of which are macrophages and some epithelial lining cells This lesion frequently gives the gross picture of organization in that it has a grayish translucency No areas of necrosis are seen in the lungs the change being en

tirely one of proliferation with a small amount of exudate Hence after the disease is over the lungs return to normal

Spleen, Liver, and Kidney Lesions in Man

The spleen shows the typical picture of a reacting spleen and the liver and kidney show parenchymatous changes the liver fre quently exhibits fatty changes and the kidney extensive cloudy swelling The lymph nodes show rather marked hyperplasia

The brain in birds shows little damage In mammals small perivascular hemorrhages and the presence of round cells in the Virchow Robbins space are common Indeed it has been claimed that at times there is a perivascular demyelinization but rather extensive investigation indicates that this does not occur There is some increase in glial cells and some disappearance of Nissl substance in the nerve cells and occasionally a necrotic nerve cell is seen The possibility that there is an endothelial prolifera tion of the blood vessels somewhat resembling the vascular lesion in typhus has been looked for by us and other investigators but so far nothing of this nature has been described The virus has not been recovered from the brain and it is our feeling that the lesions in the brain are due to the severe toxemia and not to the actual presence of the virus

Lesions in the Mouse

The mouse which is used as a test animal presents an interest ing picture when inoculated intraperitoneally with the virus The chief lesions are in the spleen and the liver The spleen is enlarged and microscopically shows a destruction of the architecture along with focal areas of necrosis The liver shows areas of focal eosino philic necrosis The virus can be obtained from both the spleen and the liver The lungs would probably show involvement if the virus were injected into the respiratory tract but show little change in mice which are injected intraperitoneally

Summary

The pathology of psittacosis is primarily one of proliferation and necrosis In birds the lesions are present primarily in the liver

and spleen In man and other mammals the predominant lesion is in the lung

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5

The Diagnosis of Psittacosis

In considering the diagnosis of psittacosis attention will be given not only to psittacosis itself but also to those diseases which are closely related to it namely ornithosis meningopneumonitis and Louisiana fever (*1*) For the most part the means of diagnosis of all four are the same and can be divided into three categories the clinical and ecologic history of the patient the recovery of the agent and the immunologic response

Clinical Examination

Characteristically the patient is very sick with a high fever and a slow pulse The onset is usually sudden with malaise chills and headache In those patients with a pneumonitis there may be a dry cough or scanty sputum but in many cases the lung involvement is first revealed by X ray examination In many instances *there is no localization even in the lungs however in all* severe cases including septicemic ones an enlarged soft spleen is said to be an important diagnostic indicator Of perhaps greater frequency are cases showing varying degrees of mildness down to those in which the infection is truly inapparent

Cases of pneumonitis of greater or lesser degree with high fever and marked toxicity or simple cases of malaise and fever should always lead the clinician to *inquire carefully into the ecologic* background of the patient. Has the patient had any recent contact with birds particularly psittacine birds or flocks of pigeons or with groups of domestic birds such as pheasants chickens or

ducks or finally with wild birds in which an unusual degree of morbidity or mortality has been noted? Is the patient by occupation a trapper or one whose livelihood takes him out into the woods and fields in closer contact with wild birds than is usual with the general run of the population? Psittacosis and ornithosis in their many variants except under very rare circumstances are acquired only by transmission from birds, but the avian human transmission can occur only too readily since dry but viable virus tends to be present in enormous quantities on the plumage of the overtly infected bird or even at times of the asymptomatic carrier

Recovery of the Agent

Suggestive clinical history and the clinical picture by themselves are very rarely sufficient to establish a diagnosis The agent may be looked for and obtained from blood sputum or in the case of fatal outcome by autopsy from organ material If the blood contains the agent it usually becomes positive in the first week of infection and isolation of the agent can best be carried out by intranasal or intraperitoneal inoculation into mice Two or three blind passages may be required if the first is negative Isolation from the blood and passage by the intraperitoneal route help the clinician to differentiate the agents of psittacosis from the related agent of lymphogranuloma venereum which has only once been reported found in the blood of an infected individual and does not produce an overt infection in mice by the intraperitoneal route

The agent of psittacosis may be found in the sputum over a more prolonged period of time and even during the early days of convalescence The sputum will almost certainly be infected with bacteria and it is therefore best to grind it lightly with a mixture of sulfonamides and streptomycin to which mixture the agent has little if any susceptibility This drug sputum mixture can be utilized for inoculation intraperitoneally into mice by which route related agents do not infect, or into the yolk sacs of seven to ten day-old chick embryos At autopsy of the mice the agent is most likely to *be found in the lung or spleen and passage may be made either* by intraperitoneal injection into mice or by yolk sac inoculation

The agent when isolated by any of the methods described

above must next be identified It has of course characteristic shape size and staining with Castaneda or Macchiavello stains but these tinctorial characteristics are shared by related agents Further identification may be carried out by test of tissue tropism as already indicated above and also by certain serologic tests which are specific

Immunologic Response

Immunologic diagnosis of psittacosis can be carried out by one of several methods The usual laboratory procedure is the complement fixation test With this test a fourfold rise in antibodies between the acute and the convalescent or late serum samples is significant as is a titer of 1 32 or higher if no acute serum sample is available It must be understood however that the complement fixation test as carried out with the antigens usually available in the diagnostic laboratory is a group specific test and that some degree of cross fixation occurs between all members of the genera *Chlamydozoa* or *Miyagawanellae* Although the test as normally performed depends upon group specific antibodies there are also type or species specific antibodies the effects of which however tend to be masked by the predominance of the group specific Absorption of serum may therefore serve to give some differentiating diagnosis between antibodies due for example to psittacosis or to lymphogranuloma More recently British and American investigators have attempted to isolate purified type specific antigens It appears that the group specific antigen which is heat stable is a carbohydrate It may therefore be treated with potassium periodate and inactivated (*2 3 4*) Treatment with HCl is said also to destroy the group specific antigens (*3*) Sigel and Pollikoff (*4*) believe that there are two group-specific antigens but that the one which is resistant to periodate can be removed by simple washing

Indicating clearly the existence of type specific antigens in the different species of the *Miyagawanellae* and giving sharp type specificity in the absence of any special prior treatment of the antigens are the neutralization test using chicken *serums (5) and* the toxin antitoxin neutralization test (*6*) In these two procedures

cross reaction is rare and occurs only in insignificant degree when it is present Unfortunately however neither test is yet adapted to routine diagnostic use

Finally there is the skin test which although well worked out in the case of lymphogranuloma venereum has been applied but little to psittacosis In the case of the lymphogranuloma antigen there is some evidence of specificity and cross skin reactions do not occur in patients with psittacosis or ornithosis with the same frequency as do the cross complement fixation reactions What little work has been done suggests that the same may be true for the psittacosis antigen but considerably more work is needed before the test could be employed to a good purpose Since in psittacosis the skin test becomes positive late if at all it might be employed particularly in the convalescent stages of the disease if certain diagnosis had not been made up to that time It is not clear whether this antigen antibody reaction in the skin is due to the same antigen antibody as partakes in the complement fixation test What little evidence there is would perhaps speak against such a possibility In any case the skin reaction becomes positive only after the complement fixation titer has risen and it disappears before the complement fixation titer returns to normal in those individuals in whom due to successful chemotherapy or spontaneous tissue sterilization the complement fixing antibodies do eventually disappear

Summary

In summary it may be said that the diagnosis that an individual is suffering from or has suffered from a disease due to an agent of the psittacosis lymphogranuloma group is not difficult to make Differentiation within the group may be more difficult, as may be the decision as to whether the acute episode is due to that particular infection or not All agents in this group tend to set up a chronic carrier state and an immunologic evidence of an infection *whether overt or latent tends to persist for years if not for life* thereby confusing the issue when an acute infection supervenes If the agent is not isolated the only certain test is the use of paired serums and the demonstration of a rise in titer fourfold or greater

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6

Psittacosis in Turkeys and Fowls as a Source of Human Infection

ORNITHOSIS (psittacosis) in man has been attributed both to psittacine and non psittacine birds of several species (*1 2*) but the source of many infections is unknown Secondary infections of man (*3 4*) have occurred Psittacosis like viruses have been recovered from animals (*5 6 7 8 9*) Reports of psittacosis cases associated with pigeons (*10*) chickens (*11*) ducks (*12*) and pheasants (*13*) and in poultry dealers (*14 15*) suggest that the importance of common barnyard birds in the spread of psittacosis frequently is overlooked Previous reports by our co workers and ourselves have concerned two outbreaks of ornithosis acquired from dressing turkeys or chickens (*16 17*) and the recovery of an agent from two turkeys in a flock (*17*) believed to have been the source of the second outbreak The present report concerns data on an outbreak of suspected psittacosis in Texas five outbreaks of psittacosis apparently acquired from dressing turkeys in Texas and a case apparently resulting from dressing chickens in Nebraska

Epidemic of Suspected Psittacosis at Malone, Texas, in January 1938

The first outbreak occurred in a rural community near Malone Texas approximately 60 miles southeast of Dallas during the second and third weeks of January 1938 Seven infections four of which proved fatal occurred one to two weeks after contact with a relative a young farmer F R whose illness also was fatal Curiously enough F R had become ill December 27 1937 about ten days after "doctoring a sick calf In retrospect these illnesses were quite suggestive of psittacosis Mrs F R had no knowledge that her husband had had recent contact with parrots or lovebirds and none of the neighbors owned one of these birds Since psittacine birds were believed to be the principal source of psittacosis the possibility that the local barnyard could have been the source of F R s infection was not considered at the time Unfortunately laboratory aids were not invoked in the study of this epidemic

Three Outbreaks of Psittacosis in a Poultry and Egg Plant at Giddings, Texas, 1948, 1951–52, and 1952

In October and November 1948 during the height of the pre Thanksgiving rush the first of three outbreaks of psittacosis occurred among the employees of a poultry and egg plant at Giddings Texas Giddings is a town of about 2 500 inhabitants located in southeast Texas between Austin and Houston in an area well adapted to raising poultry and livestock The sequence of the three outbreaks involving 85 cases with seven deaths is shown in Table I Seventy one of the 85 cases were in females Fifty three

TABLE I Incidence of psittacosis in three outbreaks in a Texas poultry plant 1948–1952 with reference to type of operation

DATE OF OUTBREAK	Killing and Picking Rooms		Evisceration Room	
	CASES	DEATHS	CASES	DEATHS
October–November 1948	22	3		
December 1951–January 1952	34	3	10	1
April–May 1952	14		5	

of the 85 cases including all of the deaths occurred in Negroes The distribution of cases by race and sex is shown in Table II

TABLE II Race and sex distribution of 85 cases of psittacosis in three outbreaks in a Texas poultry plant, 1948–1952

Date of Outbreak	Negro				White			
	Males		*Females*		*Males*		*Females*	
	Cases	Deaths	Cases	Deaths	Cases	Deaths	Cases	Deaths
November 1948	4		16	3	1		1	
December 1951–January 1952	7	2	20	2	2		15	
April–May 1952			6				13	
Total	11	2	42	5	3		29	

Description of Plant and Operations

The poultry dressing plant is a one story structure divided into five or six compartments or rooms one of which is occupied by the office The plant processes chickens turkeys, and occasionally a few ducks or guineas The firm obtains produce over a considerable part of the surrounding area and buying stations are maintained in several cities Poultry is brought in by truckers night and day during the rush season

The dressing of turkeys is a rather seasonal work with three well defined periods of activity Most turkeys are dressed during a three to five weeks period before Thanksgiving and during a shorter period before Christmas A third period of activity occurs at the end of the egg laying season in the spring when a large number of turkey hens are dressed The three epidemics in this plant were observed to fit the three periods of greatest activity in dressing turkeys

For processing on the assembly line turkeys are driven from the pens and coops in the rear of the building through a chute to the killing platform From the killing platform following the plucking of some feathers the birds are suspended on hangers and passed into the hot water bath Subsequently rubber devices remove most of the feathers In 1948 the assembly line passed through a drying oven and then into a melted wax bath After a quick dip in cold water the wax was removed along with the adherent feathers The removal of pin feathers is a tedious

process and requires many workers who perform this operation with the aid of dull knives

Both turkeys and chickens are processed on the same assembly line but in 1948 the work was done by essentially different crews Turkeys were nearly all "New York dressed" by a Negro crew and chickens were full dressed by a white crew (New York dressed" birds are picked clean but the head remains and the entrails are not removed) Unless there was an unusual rush the Negroes were laid off or assigned to clean up or miscellaneous duties while the chicken assembly line was operating The white women worked on eggs picked and eviscerated chickens eviscerated the few turkeys requiring full dress and did miscellaneous tasks The white men meanwhile bought and trucked in birds packed turkeys or chickens and did miscellaneous tasks In the Negro crew the men did the killing operated the wax machine removed most of the feathers and did clean up work The Negro women assisted in the removal of wax and adherent feathers and particularly removed the pin feathers from turkeys

After the first epidemic a number of sanitary improvements were recommended and were made A notable change was made from "New York dress" to full dress of turkeys The wax machine was eliminated and some machine operations were added in the eviscerating room Although the work of the "pinners" remained largely unchanged the assembly line procedure was streamlined considerably More eviscerating and clean up work was required because of the change in dressing turkeys It was disturbing that the sanitary and assembly line improvements did not prevent the occurrence of two more epidemics

Epidemiologic Findings in the 1948 Outbreak

As the turkeys arrive in increasing numbers each fall a point is reached when all other work is greatly overshadowed or entirely stopped in order to take care of the turkeys Prior to the outbreak in October and November 1948 the last chickens were processed on October 19 Relatively few chickens and many turkeys had been processed during the previous ten days No guineas or ducks had been handled in recent months

As the principal epidemiologic investigations were made several

weeks after the outbreak the employer s absentee records were of help in establishing dates of onset for those sufficiently ill to require time off from work The onset of the first case was on or about October 29 and that of the last case November 8 Sixteen of the 22 cases occurred between October 31 and November 4 Between October 15 and 29 new workers especially Negro women were employed almost daily to take care of the increasing number of turkeys It was found that during the week before October 29 all who became ill worked only on October 23 Twenty of the 22 cases were in Negroes A white supervisor who frequently assisted the Negroes and a Latin American woman who had been wrapping heads and frequently filling in as a pinner on turkeys accounted for the remaining two cases

The occupations of employees are shown in Table III There

TABLE III Distribution of 22 cases of psittacosis in an outbreak in a Texas poultry plant in 1948 by type of operation

DEPARTMENT	*No of Employees*	*No of Cases*
Killing and Wax Machine	6	3
Wax Removal and Picking Turkeys	32	18
Dressing Chickens	6	0
Grading and Packing	7	0
Truckers Receiving and Miscellaneous	20	1
Office	7	0
TOTAL	78	22

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were 22 cases of psittacosis with three deaths among 78 workers an attack rate of nearly 30 per cent Among the 38 workers on the turkey assembly line exclusive of the white packers the attack rate was above 55 per cent

It was evident that the struggling of the birds raised considerable dust at the beginning of the assembly line The men working in the receiving department and/or performing miscellaneous duties were obviously exposed to the dust raised around the birds however none of the illnesses occurred in these groups The feathers probably were not the source of infection either since the ma

jority of the Negro women who developed psittacosis had been in contact mainly with the pin feathers The bulk of the feathers had already been removed and in the process of removal the feathers went through the hot water and hot wax The Negro men and women obviously were exposed to the discharges from the birds It was noticeable as the pickers worked that their noses were rela tively near the vents of the turkeys and that discharges frequently exuded from the vents Furthermore the turkeys were purposelv vented thoroughly along the assembly before the birds were turned over to the white men for packing It was obvious that many of the activities contributed to the formation of aerosols Both droplet in fection and direct contact also probably were of some importance in the mode of transmission

In view of the explosive nature of the outbreak it appeared that a particular flock of turkeys which was dressed on October 23 probably constituted the source of exposure This indicated an incuba tion period of from one to two weeks in good agreement with that thought to exist in psittacosis However it was impossible to draw any conclusion as to which flock of turkeys was to be incriminated While they were processed as rapidly as possible the accumulation of the birds represented numerous flocks during a single 24 hour period

Since psittacosis frequently is a latent inapparent infection in birds it was not surprising that the workers were unable to recall the processing of any unhealthy birds In fact it was said that the turkeys had been in unusually good condition

Even admitting that some of the infections could have been ac quired from chickens it was impossible to make any inference re garding the origin of a suspect group Chickens were not dressed on October 23 Possible implication of chickens seemed highly improbable

Although the work is seasonal most of those who became ill had worked in previous years and it might be inferred that this out break represented the first effective exposure of the group to the disease There was no evidence of psittacosis in the homes or among contacts of those ill nor was there any history of any similar illness at other buying stations or on several farms from which produce was trucked to the Giddings plant

Epidemiologic Findings in the 1951–52 Outbreak

The second outbreak of psittacosis in the Giddings plant oc curred late in December 1951 and early in January 1952 There were 134 men and women engaged in dressing turkeys and chick ens during the probable period of exposure Forty four cases of psittacosis with four deaths occurred in this epidemic The date of onset of illness was determined for 42 of the 44 cases The first illness began on December 25 and the last began on January 9 Thirty eight became ill between December 27 and January 3 and 41 of the 42 cases had their onset within a 12 day period The ex plosiveness of the epidemic was highly suggestive of a common source of exposure The one case with a delayed onset possibly was a secondary case but more likely was an example of a long incuba tion period

Although some of those who had had psittacosis in the previous outbreak were working during the two weeks prior to the second outbreak there was nothing to suggest that a human carrier was the common source of infection Indeed three reinfections oc curred among those who had had psittacosis in 1948 It seemed more probable that a particular flock of birds was the source of in fection

From a tabulation of work done during the two weeks before the epidemic began it is evident that birds were dressed only on six of those days (Table IV) In searching for the day of exposure it appears that December 11 12 and 13 were too early as the in

TABLE IV Tabulation of turkeys and chickens dressed from December 11–20 1951 in a Texas poultry plant.

DATE	*Total No of Birds Dressed*	*No of Turkeys Dressed*	*No of Chickens Dressed*
Dec. 11	2231		
Dec 12	2591		
Dec 13	1671	1501	170
Dec 14	0		
Dec 15	2081	2070	11
Dec 16 17 18	0		
Dec 19	1541	1541	0
Dec. 20	1249	1163	86

* 937 of these turkeys came from Farm A (see Tables VIII and XI)

cubation period would have been too long Thus it seems that December 19 or 20 or possibly December 15 (giving a long mean incubation period of 15 days) was the critical day Fortunately some of those who became ill did not work on all three days

Careful inspection of the time cards showed that on December 15 four of the 44 individuals who became infected did not work while on December 19 two of the 44 did not work but on December 20 all the 44 worked Using this date for reference the mean incubation period was ten days with a range of five to 20 days The incubation period for 97 per cent of the cases was five to 16 days These findings fitted the circumstances Thus it appears that December 20 undoubtedly was the date of exposure The incubation period tended to be shorter for cases among workers in the picking room than among those in the eviscerating room However the shortest incubation period concerned the Negro clean up man in the eviscerating room this illness was fatal The incubation period appeared to be influenced much more by occupation and the degree of exposure than by age sex or race

One of the most interesting aspects of this epidemic was the relation of illnesses to the type of work The distribution of cases in the plant is shown in Table V Both the two men engaged in

TABLE V Distribution of 44 cases of psittacosis in an outbreak in a Texas poultry plant in 1951–52 by type of operation

DEPARTMENT	*No Presumably Exposed*	*No of Cases*	*Attack Rate (Per cent)*
Pre Picking Rooms	20	3	15 0
Picking Room	53	30	56 6
Eviscerating Room	36	9	25 0
Packing Room	19	0	0
Picking and Eviscerating Rooms	3	2	66 6
TOTAL	131	44	33 6

killing the birds and the two men who did clean up work became ill and one of each group expired Two of the three women who opened and cleaned out the crops became ill The two operators of the wing cutting and the neck cutting machines became ill Both the grader and the veterinarian in the eviscerating room became ill

Epidemiologic Findings in the 1951–52 Outbreak

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Although some of those who had had psittacosis in the previous outbreak were working during the two weeks prior to the second outbreak there was nothing to suggest that a human carrier was the common source of infection Indeed three reinfections occurred among those who had had psittacosis in 1948 It seemed more probable that a particular flock of birds was the source of infection

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Dec 14	0		
Dec. 15	2081	2070	11
Dec 16 17 18	0		
Dec 19	1541	1541	0
Dec. 20	1249	1163	86

* 937 of these turkeys came from Farm A (see Tables VIII and XI)

TABLE VII Race and sex distribution and attack rates in an outbreak of psittacosis in a Texas poultry plant in 1951–52

	White		Negro	
	Male	*Female*	*Male*	*Female*
No Workers	27	43	[illegible]0	4[illegible]
No Ill	4	13	5	22
Attack Rate	14 8	30 2	25 0	53 4

The high attack rate pointed to a widespread lack of immunity yet scarcely more than three years earlier an outbreak of psittacosis had occurred in workers in this plant In fact eight of the 22 persons who had psittacosis in 1948 apparently were exposed (as pickers or eviscerators) in 1951 Five escaped but three suffered reinfection Nine others who apparently were exposed but had escaped infection in 1948 became infected in 1951–52 Seven of the nine were pickers Two of the four deaths occurred in persons who presumably were exposed but were not ill in 1948 one was a killer and the other was a picker

Epidemiologic Findings in the April and May, 195[illegible], Outbreak

A third outbreak of psittacosis occurred in the Giddings plant in April and May 1952 when 19 cases developed There were no deaths The first two patients became ill on April 29 while the last one became ill on May 15 The remaining 16 became ill between May 2 and May 10 There was a slight possibility that the last patient an eviscerator acquired infection from her daughter who became ill on May 7 more likely this was an example of a long incubation period Eighteen of the 19 cases were in females and five of the women were Negroes All of those who became infected had been working in the picking or eviscerating rooms dressing chickens and turkeys The explosiveness of the outbreak again suggested that a single lot of birds probably was the source of infection Turkeys had been dressed on only two days during the two weeks prior to onset of the first case and all those ill had helped dress turkeys Data on plant operations are shown in Table VIII from which it is

Both of the women who worked on the gizzards became ill The absence of cases in the group working in the coolers and packing room was remarkable Likewise none of those who disposed of the feathers or who processed the entrails in the rendering plant became ill The absence of cases among the truckers was not surprising Thus nearly all the cases occurred in workers whose places on the line were between the killing operation and the end of the operations in the eviscerating room with most of the cases in pickers Both air borne infection and direct contact probably were important in the mode of infection

The ages of those ill varied from seventeen to sixty two The fatal cases were in the forty and sixty year age groups The age group distribution of cases is shown in Table VI

TABLE VI Age race and sex distribution of 44 cases of psittacosis in an out break in a Texas poultry plant in 1951–52

Age-Group	No Ill White M	White F	Negro M	Negro F	Total
17–20				1	1
20–30		2	1	4	7
30–40		1		3	4
40–50	2	4	3	5	14
50–60		5	1	6	12
Over 60	2			3	5
Unknown		1			1

The highest attack rate (43 5 per cent) and all the deaths occurred in the Negro workers This apparently was not so much an indication of greater susceptibility but rather the result of greater exposure Eighty two per cent of the Negro workers but only 51 per cent of the white workers were employed in the picking or eviscerating rooms The higher attack rate in females was believed to be indicative more of exposure than of greater susceptibility It should be noted that females were employed mainly in the picking and eviscerating rooms where the risk of exposure was undoubtedly greatest The distribution of cases by race and sex is shown in Table VII

group had worked primarily in the picking room during previous epidemics The absence of reinfections perhaps was not surprising since relatively few of those previously ill were working

Clinical Findings on the Three Outbreaks at Giddings

Records of 33 patients who were hospitalized were fragmentary and incomplete In those remaining at home the illness was somewhat similar but even less completely observed Most of those who remained at home were seriously ill although a few had mild influenza like illnesses Occasional instances of subclinical or inapparent infections were found through serologic tests on workers presumably exposed to the etiologic agent but without evidence of illness Most of the illnesses were sudden in others the onset was more insidious The illness was ushered in by vague constitutional symptoms with few if any symptoms referable to the respiratory tract The onset usually was characterized by fever anorexia chilly sensations severe headache and nausea or vomiting Unless prompt and vigorous antibiotic treatment was instituted the disease was slowly progressive and after a few days most patients felt much worse At the height of the illness the most common complaints were feverishness headache cough weakness nausea and loss of appetite Several patients complained of chills drenching sweat and pain or soreness in the chest Abnormal findings on physical examination usually were negligible at the time of hospitalization even though the patients were quite toxic usually with high fever During the height of illness the patients frequently were delirious or disoriented The most seriously ill became lethargic or stuperous this frequently was a cause for comment Relatively few patients had respiratory difficulty even if a cough was present The cough tended to be non productive X ray chest plates were made on several patients but apparently there was little correlation between the severity of illness and degree of pulmonary involvement Roentgenologists reported that the films showed pneumonitis or atypical or beginning pneumonia Because of the paucity of physical findings without the X ray examinations possible lung involvement in several cases hardly would have been seriously considered Only rarely was a rash

TABLE VIII Record of dressing operations in a *Texas poultry plant prior to* an outbreak of psittacosis in April–May 1952 with reference to the source of birds

Source of Birds	*Date Dressed*	*No of Chickens*	*No of Turkeys*
Several	April 14	5648	0
Several	April 15	4771	0
Farm A *	April 16	1251	1542
Several	April 19	3992	0
Several †	April 25	0	1657
Several	April 26	0	0

* Same source of birds which were believed incriminated in second outbreak.
† Largest flock suspected because of size and poor laying and fertility record.

seen that no birds were dressed on April 17 18 20 21 22 23 or 24 Since the incubation period averages one to two weeks it appears that one of the lots of turkeys dressed on April 25 must have been the source of infection This would indicate a mean incubation period of eight days and a range of four to 18 days Furthermore one of the individuals infected began working as a picker on April 25 Inspection of the time sheets showed that April 25 was the only day all those ill had worked with one exception that week This exception the single male infected did not work April 25 but worked in the cooler and packing room the following day perhaps significantly he also cut necks of turkeys that day The fact that this man and one of the women did not work on April 16 was important in eliminating suspicion of one farm (Farm A) as the source of infection

Most of the white women worked in the packing room when chickens were dressed but had helped dress both lots of turkeys The high attack rate among the white women apparently was due to the fact that relatively few Negro women were working The white women almost without exception were engaged in picking or eviscerating the turkeys The failure of the killer and the clean up men to become ill was noticeable Again there was little evidence of immunity in view of the fact that most of those ill had been working during one or both of the previous epidemics It should be observed however that only the Negro women as a

group had worked primarily in the picking room during previous epidemics The absence of reinfections perhaps was not surprising since relatively few of those previously ill were working

Clinical Findings on the Three Outbreaks at Giddings

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noted Lymphadenopathy was not a prominent feature The spleen or liver generally was not recorded as being palpable Abdominal distension was troublesome in the more severe cases and abdom inal surgery was contemplated but not done on two of the earlier fatal cases

The usual supportive treatment was given hospitalized patients, and the earlier cases were treated with penicillin Although the patients as a group were hospitalized or treatment was instituted rather late in the illness there was some slight evidence that penicillin was beneficial The broad spectrum antibiotics (aureo mycin terramycin and chloramphenicol) were used variously in treatment of most of the later cases with considerable evidence that all were beneficial particularly when treatment was instituted early The duration of illness varied from a few days to several weeks More than half of the patients were ill two or three weeks Several patients had relapses relapses occurred both in treated and untreated cases Those more seriously ill convalesced slowly but excepting the fatal cases recovery generally was complete and uneventful except for prolonged weakness There were four deaths in nonhospitalized and three in hospitalized cases In the fatal cases broad spectrum antibiotic therapy either was not in stituted at all or was much delayed Deaths in the earlier cases originally were attributed to widely different causes including pneumonia appendicitis and encephalitis Unfortunately autop sies were not performed

Laboratory Findings on the Three Epidemics at Giddings

Clinical laboratory findings were not noteworthy A few leu cocyte counts done either were in the normal range or showed a slight leucopenia A transient albuminuria frequently was noted Febrile agglutination tests and the heterophile and cold agglutinin tests were found to be negative

The complement fixation test as used by Shaffer and Rake (*18*) with lymphogranuloma venereum (LGV) antigen proved of great est value in the investigation of these outbreaks Differential diag nosis was not readily made by local physicians and the demon stration of a sharp rise in LGV titer in representative cases pro-

vided the first strong evidence of infection by a member of the LGV psittacosis group of viral agents

A modified Kolmer quantitative complement fixation technique was used The LGV antigen was prepared from the yolk sacs of embryonated eggs Normal yolk sac antigen control and known positive and negative serum controls were included in each series of tests Whenever possible paired or serial serums were tested simultaneously Psittacosis antigen (Lederle) also prepared from yolk sacs was used in a number of tests The results were comparable with findings obtained in the presence of the LGV (Lygranum) antigen Phenolized yolk sac antigen prepared by us incorporating feline pneumonitis virus was reactive with these serums but proved to be rather unstable

Rising titers frequently were not demonstrated because of delay in taking blood samples In the study of the first Giddings outbreak a significant rise in titer was shown in only one case but during convalescence or after recovery the majority of cases showed high titers which tended to persist for several weeks or months (Table IX) In the second Giddings outbreak a significant rise in titer was shown in 11 of 22 cases on which more than a

TABLE IX Results of complement fixation tests with lymphogranuloma venereum antigen on late paired serums in an outbreak of psittacosis in a Texas poultry plant in 1948

Case No	*Date of Onset*	*Date of Bleeding*	*Serum Dilution*				
			1 20	1 40	1 80	1 160	1 320
1	11–1–48	3–25–49	4	4	4	±	—
		3–30–50	2	—			
2	11–1–48	3–25–49	4	4	4	4	—
		6–13–49	4	4	4	3	—
		11–2–49	4	4	2	±	—
3	11–5–48	3–25–49	4	4	3	±	—
		6–13–49	4	4	3	—	—
		11–[illegible]–49	4	[illegible]	—	—	
4	Exposed not ill	10–26–49	4	4	4	3	—
		3–30–50	4	4	3	—	—
5	11–2–48	3–25–49	4	4	4	4	3
		10–26–49	*4*	*4*	*4*	*3*	±
6	11–3–48	8–2–49	4	4	2	—	
		10–26–49	4	±	—	—	

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single bleeding was done Characteristic findings with paired serums from cases in the third Giddings outbreak are shown in Table X. Significant rises in titer frequently were delayed or sup-

TABLE X Results of complement fixation tests with lymphogranuloma venereum antigen on late paired serums in an outbreak of psittacosis in a Texas poultry plant in April–May 1952

Case No	*Date of Onset*	*Date of Bleeding*	*Serum Dilution* 1 16	1 32	1 64	1 128	1 256
1	4–30–52	5–12–52	2	—			
		7–15–52	4	4	±	—	
2	5–2–52	4–9–52	—	—			
		7–15–52	4	4	4	4	4
3	5–4–52	5–15–52	2	—			
		6–24–52	4	4	4	2	—
4	5–4–52	5–21–52	4	4	4	2	—
5	5–5–52	5–21–52	4	—	—		
6	5–6–52	6–7–52	4	4	4	2	—

pressed in cases receiving early and intensive broad spectrum antibiotic therapy

Titers frequently were found in persons who were not ill. Presumably this was evidence of exposure without evidence of clinical infection. However since the test detected antibodies against the LGV psittacosis group of viral reagents possible lymphogranuloma venereum infection could not be ruled out in occasional cases In the study of the second and third Giddings epidemics serums from 12 of 37 workers who were not ill showed titers ranging from 1 12 to 1 92 Four of the 12 had psittacosis in 1948 In several instances workers became ill after their serums earlier had shown good titers

Late in the progress of each of these three epidemics blood specimens and particularly sputum and throat washings were obtained from several acutely ill persons but each attempt to recover the agent by mouse or yolk sac inoculation ended in failure It should be noted that almost without exception the patients were on intensive antibiotic therapy by the time the specimens were collected It was also disappointing that post mortem tissues were not obtainable

Findings on the Turkey Flocks

Discovery of the source of the November 1948 outbreak was not attempted because of inability to single out a suspect flock of birds although it seemed that one particular flock of turkeys dressed on October 23 must have constituted the source of infection

In view of the strong evidence that December 20 was the day of exposure of those ill in the second Giddings epidemic attention was drawn to 937 turkeys from Farm A (Tables IV and XI) which were dressed that day The next largest flock comprised 70 of the 86 chickens dressed the same day (Farm B) Nineteen turkeys dressed that day came from Farm C and 36 from Farm D the remainder of the birds comprised even smaller lots from several farms (Table IV) Blood specimens were obtained from birds on four farms for complement fixation inhibition (CFI) tests (*19*) Negative tests were obtained with serums on birds from Farm B but positive CFI tests were obtained on the other three farms (Tables XI and XII) This preliminary survey and the results of

TABLE XI Isolation of psittacosis virus and results of complement fixation tests in principal lots of birds dressed in a Texas poultry plant on December 20 1951

Farm	No of Birds Dressed		No of Turkeys Tested for Virus		Results of CFI Tests					
					Turkeys			Chickens		
	Turkeys	Chickens	Total	Pos	Total	Pos	?	Total	Pos	?
A	937	0	2	2	7	1	2			
B	0	70			2	0		5	0	
C	19		1	0	4	1		9	1	1
D	36		1	0	1		1	4	1	

Titer 1 8 or greater

a similar survey by Meyer and Eddie (*20*) on a control group of turkey flocks suggested that ornithosis was widespread However the fact remained that the explosive epidemic must have resulted from dressing birds from one farm on December 20

Attention was again focused on Farm A when it was learned that a daughter of the operator of Farm A had virus pneumonia in December 1951 her titer was 1 64 (2+) in April 1952 Considerable time was spent in searching, without success for evi

single bleeding was done Characteristic findings with paired serums from cases in the third Giddings outbreak are shown in Table X Significant rises in titer frequently were delayed or sup

TABLE X Results of complement fixation tests with lymphogranuloma venereum antigen on late paired serums in an outbreak of psittacosis in a Texas poultry plant in April–May 1952

Case No	*Date of Onset*	*Date of Bleeding*	*Serum Dilution*				
			1 16	1 32	1 64	1 128	1 256
1	4–30–52	5–12–52	2	—			
		7–15–52	4	4	±	—	
2	5–2–52	4–9–52	—	—			
		7–15–52	4	4	4	4	4
3	5–4–52	5–15–52	2	—			
		6–24–52	4	4	4	2	—
4	5–4–52	5–21–52	4	4	4	2	—
5	5–5–52	5–21–52	4	—	—		
6	5–6–52	6–7–52	4	4	4	2	—

pressed in cases receiving early and intensive broad spectrum antibiotic therapy

Titers frequently were found in persons who were not ill Presumably this was evidence of exposure without evidence of clinical infection However since the test detected antibodies against the LGV psittacosis group of viral reagents possible lymphogranuloma venereum infection could not be ruled out in occasional cases In the study of the second and third Giddings epidemics serums from 12 of 37 workers who were not ill showed titers ranging from 1 12 to 1 92 Four of the 12 had psittacosis in 1948 In several instances workers became ill after their serums earlier had shown good titers

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tests essentially were negative Abundant elementary bodies characteristic of the LGV psittacosis agents were readily demonstrable in tissue smears stained by the Giemsa or Macchiavello techniques

The agent showed a broad range of host infectivity The agent was lethal for chick embryos and killed embryos in 72 hours when large doses were inoculated in the yolk sac Infected yolk sac was toxic for mice on intravenous inoculation Surprisingly enough this virus also killed guinea pigs regularly in four to ten days when large doses of infectious mouse spleen were inoculated intraperitoneally The serums of persons who had recovered from the infection neutralized the virus poorly if at all Three laboratory acquired infections apparently resulting from egg cultured virus confirmed the impression of a high pathogenicity of the agent for man

Experimental inoculation of two turkey poults (*17*) resulted in overt infection but in four older turkeys only one became ill Sick turkeys showed marked fibrinous pericarditis and thickened cloudy air sacs The virus was recovered from the tissues of experimentally infected turkeys upon mouse inoculation

Infective tissue was sent to Dr K F Meyer for confirmatory and comparative studies The turkey strain was found to share with the Louisiana human (*21*) and the egret (*22*) strains an endotoxic component probably responsible for their high virulence for man and their broad infectious spectrum (*20*)

The psittacosis virus was not recovered from cloacal swabs or the tissues of a few turkeys chickens ducks geese and guineas obtained from several other farms including those listed in Table XI

A particular flock of turkeys was highly suspected as the source of the third Giddings outbreak This farm was located relatively near Farm A (Table VIII) in east central Texas The 572 hens from this flock were much the largest lot of birds dressed in the plant on April 25 Furthermore this was the only flock among the sources of the largest lots of birds on which poor egg production and low fertility were admitted The owner of the 572 hens said egg production had been poor and fertility practically was nil" Three of the five turkeys remaining on the farm were brought to the laboratory for autopsy One of these birds was moribund

TABLE XII Results of complement fixation tests on domestic birds from three farms (A C D) suspected as sources of an outbreak of psittacosis in a Texas poultry plant in 1948

		CFI Titers				
SPECIES	Neg	1 2 *or* 1 4	1 8	1 16	1 32	1 64
Turkey	8	2	1	1	1	
Chicken	10	1	1		1	
Duck	1	2			1	
Goose	2	1				1
Guinea	1		1	1		

dence of the illness among workers in poultry dressing establish ments at Bryan and Corsicana where some Farm A turkeys had been processed late in 1951 In April 1952 two flocks of turkeys were located on Farm A One of these flocks A 1 was the source of turkeys processed in two plants where illnesses failed to occur The other flock A 2 was a mixed flock in which turkeys from several sources had been assembled sold and processed at the Giddings plant late in 1951 An undiagnosed illness had been present in this flock (*17*) before and well after the second Gid dings epidemic Furthermore in the spring of 1952 egg produc tion and fertility in both flocks were poor A more extensive sero logic survey revealed occasional CFI titers as high as 1 128 In a total of 65 blood specimens collected from these turkeys in April 1952 20 per cent of those from the A 1 flock and 48 5 per cent from the A 2 flock were positive reactors

On April 10 1952 two turkey hens from the flock A 2 were selected for autopsy It was difficult to find sick birds in the flock at that time Both birds showed cloudy thickened air sacs brown ish fluid in the peritoneal cavity and fibrinous pericarditis Por tions of lungs air sacs kidneys spleens and livers were saved Pooled tissue suspensions from each bird were suspended in a tyrothricin sodium sulfadiazine streptomycin solution overnight before intraperitoneal inoculation in small groups of adult white Swiss mice Twelve days later survivors among these mice were sacrificed and their spleens and livers were inoculated intracere brally in other mice which died in about 96 hours Bacteriologic

this specimen came from a woman who had a severe fever and that a Weil Felix titer had been obtained by a local laboratory a tentative diagnosis of typhus had been made Subsequently complement fixation tests for typhus spotted fever rickettsial pox and Q fever and the usual febrile agglutination tests proved negative but the LGV titer was 1 64 (4+) tests on the patient s husband and four children were negative This woman had become ill in October a few days after her departure from Fairbury Nebraska where she had worked in the eviscerating room of a poultry dressing plant from September 5 through 27 1952 Aureomycin was prescribed by her physician but she refused medication her illness was prolonged and severe and characterized by remissions and relapses

It seemed highly probable that she acquired this illness from dressing poultry in the plant although an inquiry by the Nebraska state health department failed to reveal other cases The plant had 40 to 50 employees and had been processing about 4 000 chickens daily Most of the chickens were obtained within 100 miles of Fairbury although on September 25 a particularly large shipment came from South Dakota Only chickens were processed during September although turkeys were handled later

Summary

A case of possible barnyard psittacosis in a farmer was the source of infection for seven relatives in a rural community in Texas in 1937–38 five of the eight illnesses proved fatal

Five outbreaks of psittacosis with at least 96 cases and 7 deaths occurred in employees of two Texas poultry dressing establishments in 1948 1951 1952 and 1953 The majority of the cases and all of the deaths occurred in Negroes All cases had been dressing turkeys for the Thanksgiving or Christmas market or at the end of the egg laying season The illnesses varied in severity from minor influenza like attacks to severe toxemias terminating fatally The majority of cases examined showed X ray evidence of pneumonitis or "virus pneumonia The use of broad spectrum antibiotics (aureomycin terramycin and chloromycetin) appeared to be beneficial Relapses occurred frequently both in treated and untreated cases The complement fixation test was extensively

showing lesions suggestive of blackhead Although autopsy findings on the other two birds were not very suggestive of psittacosis pooled kidney lung spleen liver and air sac tissues were treated with the antibiotic mixture and inoculated into mice A couple of blind passages were attempted but the psittacosis virus was not found

Two Outbreaks of Psittacosis at El Campo, Texas, in 1952 and 1953

Several cases of psittacosis occurred in some of the workers in a poultry establishment at El Campo Texas late in 1952 and early in 1953 El Campo is 85 miles southeast of Giddings and about 45 miles from the Gulf coast Approximately 100 persons were employed at the plant during the height of activities before Thanksgiving a somewhat reduced force also dressed turkeys in December for the Christmas market Knowledge of these cases did not come to our attention until early January 1953 when the investigation was retarded because of an influenza epidemic Laboratory confirmation was obtained on eight cases and clinical data were obtained on three more Eight cases occurred near the end of 1952 or during the first week of January 1953 Three patients however became ill earlier and one a clean up man became ill Thanksgiving day Evidently there were at least two distinct outbreaks Undoubtedly some cases were missed particularly in the earlier outbreak since several of the employees were migrant workers who had already departed for Mexico for the winter

Chickens certainly were not the source of infection since this plant handled only turkeys Although turkeys obviously were the source of these infections it was impossible to single out any suspect flocks Most of the turkeys were purchased in an area within 50 miles of El Campo but many farms were represented it seemed improbable that the source of the infection was in the Farm A locality approximately 125 miles north

A Case of Psittacosis in Fairbury, Nebraska, in 1952

In November 1952 a blood specimen was received through the Amarillo Texas health department laboratory It was stated that

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used either with psittacosis or lymphogranuloma venereum antigen as an aid in the identification of the disease All attempts to re cover the virus from the cases failed A very toxic psittacosis virus was recovered from the flock of turkeys which was responsible for the second outbreak

A severe case of psittacosis apparently was the result of evis cerating chickens in a poultry establishment in Nebraska in Sep tember 1952

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Pigeons as we encounter them now either domestically reared or living in the wild state are probably derived from the rock dove of Europe and through the centuries have been partially domesticated and carried to all parts of the world where they have adapted themselves admirably to living in close association with man Domesticated pigeons are readily bred in lofts and their remarkable homing instinct has been utilized for practical purposes They have also been selectively bred to produce a great variety of plumage and flying behavior by pigeon fanciers The feral pigeons commonly inhabiting cities and farmyards have shown remarkable adaptability to the environment and an unusual capacity to maintain the species in what would appear to be an unfavorable environment for birds

Although the pigeon is one of our most numerous birds very few studies of its life history in the wild state have been undertaken Recent studies by M W Schein and D E Davis (*4*) have shown that pigeon reproduction is not restricted to one short season but that newly laid eggs are found in every month of the year in temperate climates Pigeons congregate in flocks flying together and nesting in close proximity to each other usually in inaccessible places about buildings bridges or barns Although the clutch consists of but two eggs the continuation of breeding throughout the year results in a very high reproductive rate In Baltimore the investigators found fledgling birds usually leave the nest in about 45 days and about 29 per cent of the eggs survive to fledge in the first half of the year The fact that pigeons congregate in flocks and nest together through all months of the year may have a direct bearing upon the persistence and maintenance of infections with the virus of the psittacosis lymphogranuloma group It is worthy of note that avian infections with this virus are most prominent in birds which flock and nest together such as parakeets in their native Australia fulmar petrels and as recently shown domestic ducks and turkeys

The Incidence of Infection in Pigeons

Numerous studies in this country and Europe have shown that pigeon flocks almost without exception contain a certain proportion of individuals infected with this virus In the United States

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7

Psittacosis in Pigeons

It has been known since 1940 by the investigations of Coles in South Africa (*1*) and Pinkerton and Swank in the United States (*2*) that pigeons *Columba livia* are sometimes infected with a virus belonging to the psittacosis lymphogranuloma group This infection was soon incriminated as a source of human disease by Meyer *et al* (*3*) who isolated the same virus from a flock of racing pigeons and from the lung of a human who developed a fatal pneumonitis following exposure to these pigeons Since then it has been shown repeatedly that birds of this species both domesticated and feral are carriers of the virus and a source of dis ease in man This is not surprising in view of our present knowl edge that representatives of this group of viruses are widely parasitic among many orders of birds and mammals

The term ornithosis has been suggested for the disease caused by this type of virus in birds other than psittacine However it would be my preference to employ the word psittacosis when re ferring to the human disease caused by a virus of this group which *presumably* had its origin in any avian species Since it is often difficult to trace the actual source of the infection to a parrot a parakeet a pigeon a turkey or some other species of bird it would seem less confusing to designate the clinical disease in man as psittacosis Furthermore the pathology and pathogenesis of the disease in man are similar no matter what the source of virus and the etiologic agents themselves are quite similar though dif fering in some ways

developmental stages for the presence of virus White mice were inoculated intracerebrally with suspensions of individual embryonated eggs and other mice were inoculated intraperitoneally with suspensions from pools of four eggs each in order to provide a larger inoculum No virus was recovered from these eggs and thus no evidence for congenital transmission was secured at this time

Pathology of the Disease in Pigeons

In the previously mentioned surveys of apparently healthy birds autopsy examinations have shown relatively few abnormalities In our studies of several hundred birds which were not sick at the time of capture the only significant finding was an enlarged and friable spleen in about half the birds From only a small proportion of these was virus recovered

Pigeons are sometimes observed with obvious signs of disease and the clinical and pathologic picture of the disease in pigeons is often complicated by *Salmonella* infections and also by an unrelated virus causing intranuclear inclusions (*16*) No typical syndrome in sick infected birds has been defined but young birds are usually thin feeble and undersized Diarrhea is common and the vent feathers are matted with droppings Adults occasionally show weakness diarrhea or opisthotonos and may be found dead without presenting premonitory signs

Pigeons that succumb to the disease particularly young ones may present on autopsy an acute or subacute inflammation of the serous membranes with a dry fibrinous exudate covering the pericardium and peritoneum In older birds and in those with a latent infection the liver may be enlarged congested and with necrotic foci and the spleen is usually enlarged and friable Pulmonary lesions have not been noted consistently and are probably not part of the disease process in birds Histologically the affected serous surfaces reveal intense inflammatory infiltration with large mononuclear and lymphocytic cells predominating In the liver spleen and kidney there is vascular congestion and the parenchymatous cells show necrotic changes Impression smears of the exudate or spleen appropriately stained frequently show the virus particles

active virus has been recovered from pigeons in Chicago (5) Baltimore (6) Washington D C and Birmingham Alabama (7) and California (8) in percentages varying from 4 to 22 per cent of birds examined In Canada similar results have been obtained in cities of Ontario (9) In Paris Lepine (*10*) has demonstrated infection by serologic studies and virus isolation In England infection in pigeons was reported in 1943 (*11*), and Hughes (*12*) has described in detail the disease occurring in a pigeon flock He emphasized the manifestations of the disease in young pigeons and points out the relationship of the incidence to the breeding activity of the flock Reports from Holland by Dekking and Ruys (*13*) indicate widespread infection among pigeons in that country During World War II on orders of the German occupation forces all pigeons were destroyed Following the war according to Dekking and Ruys among pigeons imported from England and Belgium some were noted to be sick They point out that this illustrates the impossibility of eliminating the infection by destruction of birds since those imported later will undoubtedly be infected Other reports indicate that flocks of domesticated loft or racing pigeons also are frequently infected with the latent disease It appears therefore that infection with this virus is ubiquitous among pigeons and that the virus has successfully parasitized this species quite permanently

Evidence has been presented that young parakeets in commercial aviaries acquire infection in the nest probably from the adult birds and it might be assumed that this is a general mode of transmission of the infection for all birds (*14*) The transovarian or congenital transmission of infection through the egg however remains a possibility and could explain readily the wide distribution of the virus Experimental studies in our laboratory have shown that chicken eggs inoculated with a sublethal dose of virus during embryonic development will hatch and virus can be recovered for as long as 22 days from the chicks although they appear normal otherwise (*15*) The conditions of this study did not warrant the conclusion that congenital transmission in the chicken a relatively resistant species could occur but the possibility of this mode of transmission in more susceptible species remains During the spring of 1949 we examined a total of 70 pigeon eggs in all

(19 20) with satisfactory results Dr M R Hilleman in his discussion of this paper will develop this aspect of the problem

Human Psittacosis of Pigeon Origin

The clinical picture of human psittacosis known to originate from pigeons is similar to the disease known to originate from psittacine birds However it is stated (21) that the disease is inclined to be less severe and to have a lower fatality rate Now that the broad spectrum antibiotics are being widely and apparently successfully used it is doubtful if further data on this point will be forthcoming

It has been shown experimentally that pigeon strains of the virus are susceptible to therapy by the broad spectrum antibiotics and this has been found true in clinical practice (8) However it appears clear that the action is virustatic rather than virucidal since a virus can readily be recovered from surviving animals Other studies have shown the failure of these antibiotics to prevent the carrier state (8 22) Clinically it is a common experience for the patient to suffer a relapse some days or weeks after an apparently successful response to therapy

A review of the literature indicates that most of the human cases of pigeon origin can be traced to loft pigeons There are relatively few cases which are directly attributable to contact with feral pigeons and these individuals have usually had close contact with the wild birds There are other cases which have a history of exposure to the dust of pigeon roosting or nesting areas without direct contact with the birds Boucher and Sautter (23) recount a recent outbreak of seven such cases in a military camp in France These cases were all mild and occurred over a period of three months in men living to the windward side of cages containing pigeons and chickens The author suggests that the dried bird droppings carried by the wind were the source of infection

It has been pointed out (24 25) that a significant proportion possibly 10 per cent of clinical disease known as pneumonitis or primary atypical pneumonia is probably due to virus of the psittacosis group Many cases are seen in clinical practice which are diagnosed serologically as psittacosis but for which careful epidemiologic investigation fails to reveal an avian source of virus

The Virus

The virus can be readily isolated from the organs or cloacal con tents by inoculation into white mice intracerebrally or intranasally or sometimes by inoculation of the yolk sac of chick embryos Like the virus recovered from psittacine birds it forms typical clusters of elementary bodies in infected cells and appears to be identical to it morphologically and tinctorially With a few exceptions strains of virus isolated from pigeons appear to be similar to each other and all are pathogenic for mice by intracerebral inoculation They differ from psittacine strains in their reduced pathogenicity for mice upon intraperitoneal inoculation and rarely cause the death of the mouse by this route Neutralization tests with chicken antiserum (*17*) and the toxin neutralization technique (*18*) in dicate antigenic differences between the strains of pigeon origin and those of psittacine or mammalian origin

Diagnosis of Infection in Pigeons

At the present time the isolation of virus from the organs of pigeons remains the most accurate method for the determination of infection This has the disadvantage however of time consum ing animal inoculation is a potential hazard to workers and may result in failure to recover virus actually present Several mouse passages may be necessary to establish the infection in mice and the use of small inocula for intracerebral and intranasal routes is a limiting factor Larger quantities of inocula may be injected intra peritoneally into mice and followed by serial intracerebral passage for final diagnosis The demonstration of typical clusters of ele mentary bodies in impression smears of the brain stained by Mac chiavello s method from mice dying from three to seven days after inoculation is generally accepted as diagnostic Confirmatory identification may be made by preparing an antigen in embryo nated eggs and demonstrating its ability to fix complement in the presence of known positive antiserums

It has been the experience of numerous workers that the com plement fixation test using pigeon serums by the method usually employed for human serums gives irregular and equivocal results Recently the indirect complement fixation test has been employed

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Furthermore it is highly probable that there are many cases of psittacosis in man which are unrecognized because of the lack of association with psittacine birds or pigeons and the failure to attempt a serologic diagnosis This raises the question of prevalence of the disease which might be contracted unknowingly by the patient without direct contact with birds The numbers of pigeons which are about the streets parks and elevated railway platforms in cities may constitute some hazard to individuals who come in contact with the dust from the dried droppings Such an unrecognized source may be the origin of a certain number of cases although admittedly this number is small

Nevertheless the relative rarity of pneumonitis in the populations of cities suggests that there is no undue public health hazard to the population from these indirect contacts This fact together with the known significant proportion of infected pigeons suggests that there must be natural barriers to the infection It may be that the birds although infected and harboring the virus in their organs do not excrete it in their droppings except under special conditions The virus may not be as resistant to climatic conditions as is sometimes thought Or it may be that it does not reach the human lung in sufficient dosage to cause the disease

Finally there appears to be no evidence that widespread epidemic respiratory disease is due to virus of pigeon origin but localized outbreaks and sporadic cases among persons exposed to the dust of this species of bird or in intimate contact with them indicate a definite hazard Persons under these circumstances should be aware of the risk

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cytes and the allantoic fluid from embryonated eggs infected with meningopneumonitis virus (5)

Three different kinds of antigen were employed in the com plement fixation and the conglutinating complement absorption tests The first was an infected yolk sac preparation in which the antigenic activity was enhanced by treatment with phenol (2) The second was an elementary body preparation purified by ether fractionation and differential centrifugation (9) The third was a lipoidal antigen purified from ether extracts of the virus by frac tionating with organic solvents (3 4) Antigens prepared from lymphogranuloma venereum mouse pneumonitis feline pneu monitis meningopneumonitis ornithosis and psittacosis viruses were tested

Table I shows the results of indirect complement fixation tests performed with serums of chickens immunized against the orni thosis feline pneumonitis and mouse pneumonitis agents Phenol enhanced purified elementary body and purified lipid antigens

TABLE I Results of indirect complement fixation tests of rooster antiserums using phenol-enhanced purified elementary body and purified lipid antigens

ROOSTER ANTISERUM	ANTIGEN											
	Phenol enhanced						Purified elementary body					
	LV	*Mou pn mo*	*F l pneumo*	*Orn th (Men g)*	*Orn th (P4)*	*Psitt*	LV	*M us pneumo*	*F l pneumo*	*Orn th (Men g)*	*Orn th (P4)*	PUR D LIPID LV
Ornith (Kam)	80	80	80	40	80	40	40	40	40	40	40	80
Ornith (Meningo)	20	20	20	10	40	20	10	10	10	10	10	10
Feline pneumo	20	40	20	10	40	20	20	10	40	20	20	40
Mouse pneumo	160	160	160	160	320	80	80	80	80	80	80	320
Normal mouse brain (Control)	0 †	0	0	0	0	0	0	0	0	0	0	0
Normal mouse lung (Control)	0	0	0	0	0	0	0	0	0	0	0	0

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Numbers are reciprocals of serum titers

† (0) indicates that there was no inhibition of complement fixation by a 1 5 dilu tion of serum the lowest dilution tested

BY MAURICE R HILLEMAN, PH D

8

Serologic Procedure for Detecting Psittacosis Infection in Birds

THIS paper will be concerned with details of serologic procedure for detecting psittacosis infection in birds and will pre sent a brief review of some of the work carried out along these lines in the Department of Virus and Rickettsial Diseases of the Army Medical Service Graduate School (*1–6*)

Our laboratory has concerned itself with the development of procedures for (a) detecting antibody against viruses of the psitta cosis lymphogranuloma venereum group in the serums of mam mals and birds and (b) specific differentiation of the individual viruses of the group The complement fixation serum neutraliza tion conglutinating complement absorption and hemagglutina tion inhibition techniques were used

Two kinds of complement fixation procedure were utilized The first was the ordinary or direct complement fixation method the second was the indirect complement fixation test which measured the blocking or complement fixation inhibitory antibody commonly found in serums of ducks chickens turkeys and certain pigeons (*5 7 8*) The serum neutralization test was employed to measure the virus infectivity neutralizing capacity of chicken serums The conglutinating complement absorption test measured complement fixing antibody but employed a non hemolytic complement In this test a sheep-cell conglutinating system replaced the hemolytic system employed in the ordinary technique Finally the hemag glutination inhibition tests were performed using mouse erythro

TABLE III Results of complement fixation tests of pigeon ornithosis serums using phenol-enhanced purified elementary body and lipid antigens

	Antigens										
	Phenol enhanced						Purified Elementary Body				
Pigeon Number	LV	*Mouse pneumo*	*Feline pneumo*	*Ornith (Meningo)*	*Ornith (P4)*	*Psitt*	LV	*Mouse pneumo*	*Feline pneumo*	*Ornith (P4)*	Purid Lipid LV
39 A	0	0	10	0 †	0	5	0	0	20	80	ND ‡
41 B	40	20	80	40	40	80	10	10	80	160	40
28 A	20	10	20	10	10	∠0	20	20	80	160	ND
20 A	*40*	*40*	*80*	*40*	*80*	*80*	*20*	*20*	*40*	*160*	*40*
19 A	80	80	160	40	80	80	80	40	80	160	80
3740	160	80	80	80	80	80	160	80	160	320	160

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Numbers are reciprocals of serum titers

† (0) indicates no fixation of complement at a 1 5 dilution of serum the lowest dilution tested

‡ ND = Not done

TABLE IV Conglutinating complement absorption tests with various antigens and antiserums

Sera			Antigens				
Host	Vs	No	LV	*Feline pneumo*	*Mouse pneumo*	*Ornith (P4)*	*N yolk sac*
Human	Clinical diagnosis						
	LV	1	160	3∠0	160	80	0
	LV	2 ‡	>1280	>1280	640	640	160
	Psittacosis	3	640	1280	160	320	10
	Ornithosis	4	1280	640	3 0	160	0
	Normal (Control)	5	20	10	10	0	0
	Normal (Control)	6	10	0	0	0	0
	Normal (Control)	7	0 †	0	0	0	0
Pigeon	Natural infect with						
	Ornithosis	36 A	160	1280	0	160	0
	Ornithosis	31 A	20	80	0	10	0
	Ornithosis	27 A	160	>1 80	80	160	0
	Ornithosis	32 A	>1_80	>1280	80	160	0

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Numbers are reciprocals of serum titers

† (0) indicates that there was no absorption of complement by serum diluted 1 10 the lowest dilution tested

‡ Human LV serum 2 was not anticomplementary when diluted 1 40 None of the remaining sera was anticomplementary when diluted 1 10

were used It is seen that the titer of a given chicken serum was essentially the same when tested with the three kinds of antigens prepared from the different viruses Thus the indirect complement fixation reaction was group specific and failed to differentiate these agents

These results were in contrast to those obtained in serum neutralization tests with the same serums (shown in Table II) In

TABLE II Neutralization tests in mice

Test Virus	Rooster Antisera		
	Ornith (Meningo)	*Feline Pneumo*	*Mouse Pneumo*
Ornith (Meningo)	*1 03*	0 08	0 17
Feline pneumonitis	0 04	*1 18*	†−0 19
Mouse pneumonitis	0 12	0 09	*1.22*

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* Neutralization value equals mean infectivity score for normal serum minus mean infectivity score for immune serum

† (−) Indicates that the score obtained with immune serum was greater than that for the normal serum

these tests a neutralization value of 0 2 or greater was significant It is seen that homologous neutralization values of 1 03 1 18 and 1 22 were obtained There was no heterologous neutralization and the tests were highly species specific

Table III summarizes the results of direct complement fixation tests performed with serums from pigeons naturally infected with ornithosis virus In the tests with phenol enhanced and purified lipid antigens the titer of a particular pigeon serum was the same irrespective of which virus was employed hence the tests with these antigens were group specific There was however a suggestion of species specificity in the tests performed with the purified elementary body antigens and certain of the pigeon serums Thus the serum titers of pigeons 39A 41B 28A and 20A were consistently higher when tested with homologous ornithosis virus than when lymphogranuloma venereum or mouse pneumonitis antigens were used Intermediate titer values were obtained with the feline pneumonitis antigen Similar tests with serums from human cases of psittacosis ornithosis and lymphogranuloma venereum were entirely group specific and failed to reveal titer differences when the different antigens were employed

and the pigeon ornithosis serums all inhibited hemagglutination by the meningopneumonitis hemagglutinin Thus the test was group specific Nonspecific hemagglutination inhibition in a dilu tion of 1 20 or less of serum was observed in nearly all serums tested The hemagglutination inhibition procedure is simple is highly sensitive and has the advantage of detecting complement fixing or complement fixation inhibitory antibody in bird serums thus both kinds of antibody may be detected in a single test The test has the disadvantage that the antigens prepared to date have not been stable on storage

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The results of the conglutinating complement absorption tests employing phenol enhanced antigens are given in Table IV This test has not proved satisfactory in our hands because the anticom plementary titers of the antigen preparations usually approached the antigen titer For this reason the antigens had to be used in very high dilution Group specificity was revealed in the tests with human serums The results of the tests with pigeon serums were somewhat erratic The titer differences obtained are believed to reflect the inadequacies of the test more than to reveal antigenic differences between strains

Hemagglutination inhibition tests were performed with chicken and pigeon serums and the results are given in Table V It is seen that the serums from chickens immunized against ornithosis meningopneumonitis mouse pneumonitis or feline pneumonitis

TABLE V Agglutination inhibition tests with meningopneumonitis hemag glutinin (C F titers included for comparative purposes)

Antisera			Serum Titers by	
Host	*Prepared vs*	*No*	*A I test*	*C F test*
				INDIRECT TEST
Rooster	Ornith (KAM egg line)	3203	320 †	80
	Ornith. (KAM egg line)	3276	640	20
	Ornith (Meningo)	3403	640	10
	Ornith. Meningo)	3404	320	20
	Mouse pneumonitis	3101	640	160
	Mouse pneumonitis	3102	80	40
	Feline pneumonitis	3001	160	20
	Feline pneumonitis	3002	40	0
	Normal mouse lung	3503	10	0
	Normal mouse brain	3501	10	0
				DIRECT TEST ‡
Pigeon	Ornithosis (Natural infection)	43A	160	80
	Ornithosis (Natural infection)	13A	80	40
	Ornithosis (Natural infection)	28A	80	20
	Ornithosis (Natural infection)	27A	40	40
	Presumably normal (Controls)	277	20	0
	Presumably normal (Controls)	282	10	0
	Presumably normal (Controls)	298	20	0

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These tests were performed using LV antigen of the phenol enhanced type

† Numbers are reciprocals of serum titers

‡ None of these sera was anticomplementary in a dilution of 1 10 nor did any of these sera react with normal control antigen

Monday before Thanksgiving Day The following day she killed and dressed the bird and it was eaten on Thursday The patient became ill within the next two days

The New York State focus was first suggested through the discovery in April 1945 of several cases of atypical pneumonia by Dr William Wolins (2) among patients in his practice in Suffolk County Long Island The clinical nature of the illnesses and the intimate association that the patients had had with ducks led Dr Wolins to send blood specimens to the George Williams Hooper Foundation to be studied for serologic evidence of psittacosis Several of these serums showed marked fixation of complement with psittacosis antigen Obviously additional more extensive and detailed studies of this situation were needed At this point epidemiologists from the New York State Department of Health were brought into the investigation and the findings presented below represent the joint efforts of that team Dr Wolins and the Hooper Foundation

Illness in Duck Handlers Due to Psittacosis

The first step taken in the Long Island investigation was an attempt to locate additional recent human cases of psittacosis in the area This was done through the questioning of practicing physicians in the county and the search of hospital records A total of 19 cases clinically describable as atypical pneumonia was uncovered Seventeen of the patients had experienced onset of symptons during April May or June of 1945 This group probably represented a grossly selected sample of all such patients in the county since the case finding efforts were directed primarily toward populations involved in duck handling

Data on these patients relating to their color age sex residence date of onset serologic findings and association with ducks are presented in Table I It will be noted that the patients were all adults and most of them had had intimate contact with ducks possibly a reflection of the case-finding procedure The serologic evidence for the diagnosis of psittacosis is almost unequivocal in some patients highly suggestive in others and doubtful or negative in the remaining The possibility of lymphogranuloma venereum infection particularly in the six Negro individuals seemed

ROBERT F KORNS, M D

9

Psittacosis in Ducks and Persons Exposed to Ducks *

SINCE 1941 attention has been focused on the duck as a possible disseminator of virus of the psittacosis lymphogranuloma venereum group In the serologic examination of 24 domestic ducks from Michigan in that year seven showed definite though low re actions by the complement fixation test On the other hand study of serums from 55 wild ducks gave completely negative results (*1*) Although it is known that this test is not satisfactory for duck or chicken serums the results were of such a nature that further study was warranted Unfortunately tissue specimens from these birds were not available for viral isolation

The first indication that the duck might serve as a source for human psittacosis like infection appeared almost simultaneously in New York State and California In California two isolated cases of atypical pneumonia were identified as psittacosis by comple ment fixation tests In one of these with onset in July 1945 the only birds to which the patient had been recently exposed were mallard ducks and a viral agent of the psittacosis lymphogranu loma venereum group was recovered from one of the ducklings which had died The other patient whose illness began in Novem ber 1945 reported that she had been presented with a duck on the

The author wishes to acknowledge the essential help of Dr Karl F Meyer and Miss Bernice Eddie of the George Williams Hooper Foundation, who supplied the laboratory evidence referred to in this paper The contribution of Dr William Wolins who initially suggested the presence of psittacosis in the Long Island duck population is also gratefully acknowledged

unlikely based on clinical and epidemiologic information Sputum samples from acutely ill patients were not available for virus isolation and other study

The illnesses were in general mild although leading to hospitalization Most patients had fever 102 to 103° F lasting seven to 14 days with a nonproductive cough and general malaise Auscultation revealed fine rales but no signs of consolidation in the lungs On the other hand X ray uniformly demonstrated a patchy pneumonitis most prominent in the area of the hilar and main stem bronchi Further clinical details including the response to penicillin therapy in the patients considered to have had psittacosis have already been published (2)

Serologic Survey of Human Population

Although there was an apparent concentration of human cases of psittacosis in duck handlers this observation did not necessarily implicate the duck as a disseminator of the disease It should be noted that 12 of the 19 patients also had had significant contact with chickens three with pigeons and two with sea gulls all possible reservoirs of this group of viruses Furthermore since this small area produces 7 000 000 ducks a year for the consumer market a large part of the human population has close contact with these birds and it might be assumed that the association of psittacosis with duck handlers was merely fortuitous In addition the concentration of four of the cases on one small farm and three on another raised the question of person to-person transmission as the mechanism for spread of the infection

One way of assessing the significance of the association between duck handling and human psittacosis infection was to test for the presence of psittacosis antibodies in the serums from a large group of persons with or without contact with ducks Thirteen duck workers were bled from one farm where clinical cases of the disease had been present All specimens showed some complement fixation with psittacosis antigen seven of these had titers greater than 1 16 (4+) Seven of 11 blood samples from another farm where cases had occurred showed positive complement fixation tests of this titer or greater Most of these individuals did not recall any past illness of the type under study Twenty two additional serums

TABLE I Case histories of patients with clinical atypical pneumonia discovered in a survey in Suffolk County New York, in 1945

CASE NUMBER	*Color*	*Age*	*Sex*	*Residence*	*Date of Onset in 1945*	*Day of Illness Blood Obtained*	*Titer of Comp Fixation to Psittacosis Antigen (4+ Reaction)*	*Contact with Ducks*
1	W	44	M	Jamesport	4/2	23	1 32	Intimate
2	W	42	M	C Moriches	4/15	12	1 16	
						20	1 16	
3	W	49	F	"	4/29	7	1 16	
						14	1 64	
4	W	36	M		5/15	7	1 8	
						30	1 8	
5	C	44	F		5/17	10	1 128	"
						20	1 256	
6	W	47	F		5/19	16	1 64	
7	W	48	F		5/22	12	Neg	
						25	1 16	
8	C	31	F		5/31	7	1 8	"
						20	1 128	
9	C	71	F	Hampton Bays	6/12	6	1 32	None
10	C	29	F	C Moriches	6/21	4	1 8	Intimate
						14	1 16	
11	W	74	F	Riverhead	4/26/45	16	1 4	Intimate
						101	1 2	
					3/2/46	7	1 8	
12	W	45	F	C Moriches	5/5	7	1 8	"
13	W	27	F		5/21	35	1 8	
14	W	45	M	Riverhead	6/1	Early	Neg	
15	W	57	M	C Moriches		14	Neg	Occasional
						30		
16	W	47	F	Westhampton	6/6	10	1 2	None
						30	Neg	
17	W	42	F	Eastport	6/17	14	1 2	Intimate
18	C	25	M	C Moriches	6/18	4	1 4	Occasional
19	C	20	F		8/5	10	1 16	Int mate
					March	8 mo prior	1 4	"
					1946	7	1 8	

viously the duck seemed the most likely source The next step which actually had been initiated before the complete results of the serologic survey were available was to examine the ducks themselves for the presence of psittacosis virus The arduous job of collecting suitable specimens from a large group of birds in Long Island for virus isolation by a distant laboratory in California presented problems which involved all of the participating investigators in visits to the duck farms themselves Actually many of the ducks studied were obtained from farms where the disease in humans was not known to exist A sample of both healthy and sick ducks of different ages from nine farms was collected Autopsies were performed with care so as to avoid cross contamination between tissues of different organs of the same bird and between different birds Specimens were frozen immediately with dry ice and retained in this condition until tested in the laboratory

The observations presented in Tables III IV and V represent the findings of laboratory workers at the Hooper Foundation An

TABLE III Isolation of the psittacosis virus from ducks and other birds collected from duck farms in Suffolk County New York, in 1945

MONTH OF COLLECTION	*Type of Bird*	*Number Tested*	*Number Virus Isolated*	*Per cent Positive*
July	Pigeon	2	2	100 0
	Sea Gull	[illegible]	1	50 0
	Peking Duck	4	2	50 0
September	Peking Duck	15	7	46 6
October	Peking Duck	97	31	31 9
	Sea Gull	5	2	40 0
	Crow	1	0	0 0

ornithosis or psittacosis virus of moderate pathogenicity was recovered on intraperitoneal inoculation of mice from 40 (34 5 per cent) of 116 ducks studied and from five (55 9 per cent) of nine sea gulls and pigeons collected from these same farms Presence of infection was demonstrated in birds collected in July September and October Of particular interest was the recovery of virus from ducks of all ages the youngest duckling being only four days old. Virus was obtained from sick and well ducks with equal

were obtained from workers on six other farms five from chicken and turkey farms 17 from patients at the Southampton Hospital admitted for an unassociated illness 18 from coast guardsmen stationed in the area and a number from miscellaneous residents of Riverhead village including physicians nurses and merchants

In order to allow proper comparison the serologic findings have been divided into two categories showing complement fixation in a titer either greater or less than 1 16 (4+) These data for patients and healthy individuals both with and without contact with ducks are presented in Table II It is apparent that among the

TABLE II Complement fixation to psittacosis antigen in serums from cases of atypical pneumonia and from healthy individuals according to association with ducks in Suffolk County New York in 1945

Titer of Complement Fixation	Contact with Ducks			No Contact with Ducks			Unknown	Total		
	Cases	*Non Cases*	*Total*	*Ca es*	*Non-Cases*	*Total*	*Non Cases*	*Cases*	*Non Cases*	*Total*
Less than 1 16 (4+)	8	28	36	1	57	58		9	85	94
1 16 (4+) or greater	9	17	26	1	2	3	1	10	20	30
Total	17	45	62	2	59	61	1	19	105	124
Per cent with C F Titer of 1 16 (4+) or greater	53 0	37 8	42 0		3 4	4 9		52 5	19 1	24 2

group of 62 duck handlers studied the serums from 53 per cent of 17 clinical cases and 38 per cent of 45 healthy individuals showed complement fixation to psittacosis antigen in titers of 1 16 (4+) or greater This is strikingly different from the observations on the 61 persons who had no contact with ducks Only 4 9 per cent of the specimens from these individuals showed complement fixation in the same titer

Virus Isolation from Ducks

The concentration of positive serologic findings in blood samples from duck handlers of course suggested the presence of a reservoir of psittacosis infection in the working environment and ob

Discussion

The significance of these findings as related to psittacosis in either ducks or the human population associated with ducks is not clear The indirect evidence that the handling of ducks infected with a psittacosis virus may lead to occupational or to latent infection in man is quite convincing However until the virus is isolated from the sputum or blood of a patient the proof for the existence of the duck to man infection chain is incomplete Furthermore even though there is a tendency to find virus relatively more frequently in the organs of ill ducks and relatively more often in the stool of healthy ducks the virus is not found frequently enough in sick ducks to allow the conclusion that the sickness and death are primarily caused by psittacosis Certainly one should not casually assume that this virus is responsible for the serious epizootics which sporadically affect the duck industry

The concurrent existence of certain bacterial infections in the ducks studied tends to confuse further the question of the relationship between the virus and illness Thus the organs and intestines of 23 of 97 ducks cultured before being tested for virus yielded *Salmonella typhimurium Pasteurella multocida* and *Pfeifferella anatipestifer* were found in two other instances Anatomical lesions in the liver and spleen were bacteriologically sterile in six specimens and infected with *Salmonella* in four others which subsequently produced psittacosis in mice As in psittacosis of pigeons clinically normal ducks had no gross anatomical lesions despite the presence of virus in the organs The coexistence of *Salmonella* and psittacosis virus in the organs of a four day old duckling suggests the likelihood of liberal exchange of infectious agents discharged in the feces

Whether the presence of extensive inapparent psittacosis infection in the Pekin ducks of Long Island represents a significant hazard to the exposed human population is difficult to assess Certainly study of the official records on reported cases of pneumonia from this area fails to reveal any special problem In fact the experience of Suffolk County does not differ from that in other parts of New York State Review of the data accumulated through

frequency although in the sick birds recovery from the organs (*spleen liver kidney*) *was relatively more frequent than recovery* from intestinal content Thirty two (40 2 per cent) of 65 duck serums tested in the complement fixation inhibition test gave positive reactions (*3 4*)

TABLE IV Incidence of psittacosis virus in ducks collected in October 1945 in Suffolk County New York, by age of the duck

Age of Duck	*Number Tested*	*Number Virus Isolated*	*Per cent Positive*
1 wk.	5	1	
2 "	5	3	
3 "	15	6	34 8
4 "	18	5	
5 "	2	0	
6 "	8	3	
7 "	1	1	44 5
8 "	7	4	
2–7 mos	32	7	22 2
1–2 yrs	4	1	
TOTAL	97	31	31 9

TABLE V Incidence of psittacosis virus in ducks collected in October 1945 in Suffolk County New York, by health status of the duck and site of isolation

Health Status of Duck	*Number Tested*	Number Virus Isolated *From Organs*	Number Virus Isolated *From Intestines*	*Total Per cent Positive*
Healthy	33	4	7	33 3
Sick	53	15	2	32 0
Dead	11	2	1	27 3
TOTAL	97	21	10	31 9

On the other hand serologic examination of 61 comparable individuals from the same area who had not had contact with ducks yielded only three (4 9 per cent) positive in this titer

4 The virus was present to an equal extent in healthy and sick ducks and was present in ducks of all ages the youngest being four days old

5 It seems likely that psittacosis infection for the most part clinically inapparent is endemic in this duck population The extent of its role in producing illness in either the ducks or in the associated human population has not been accurately defined However it is doubted that the hazard to humans is a problem with which one should be overly concerned

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4 Meyer K F Ornithosis and psittacosis In *Diseases of Poultry* ed by Biester and Schwarte (Ames Iowa Iowa State College Press 1953) pp 569–618

extensive mass chest X ray surveys in this county fails to indicate an undue number of pulmonary abnormalities Inquiry of the duck handlers themselves does not reveal historical evidence of a special problem although some of the older farmers recall annual bouts of a febrile respiratory illness following the handling of the accumulated dry duck feathers Since then however the procedure has been changed so that the feathers are always wet during this operation These farmers indicate that the respiratory illnesses seen in former years no longer occur

The available facts suggest that on the farms studied a delicately balanced parasitism exists between a psittacosis virus of low virulence and the duck population For that matter the presence of infection in sea gulls and pigeons from these same farms widens the scope of the bird reservoir involved It seems likely that the distribution of infection noted in the observations of 1945 represents only a brief moment in the prolonged endemic occurrence of the disease in these birds The normal annual practice on these farms is to slaughter 99 per cent of the 7 000 000 ducks produced retaining only 1 per cent as brooders Even this drastic action has apparently failed to eliminate the infection

Undoubtedly it would be of interest to reassess the status of psittacosis infection on these farms at the present time a decade after the original studies It is quite possible that the findings would be similar Nevertheless it is doubted that the hazard to the human population is a problem with which one should be overly concerned

Summary

Study of Pekin ducks from commercial duck farms in Long Island New York and of the human population of that area in 1945 has produced the following findings

1 A psittacosis virus of moderate pathogenicity was recovered from 40 (34 5 per cent) of 116 ducks examined

2 Human cases of atypical pneumonia occurring in duck handlers were identified as psittacosis by serologic tests

3 Examination of serums from 62 persons who had close contact with ducks showed 26 (42 per cent) with positive complement fixation with psittacosis antigen in a titer of 1 16 (4+) or greater

Incidence of Psittacosis in Wild Pigeons

Our interest in feral pigeons was aroused when we isolated a virus of the psittacosis group which has been called the Illinois virus from two patients with fatal cases of pneumonitis (4) Neither patient had any known contact with psittacine birds or with persons infected with psittacosis Since it was reported that at least one of them had enjoyed watching wild pigeons we decided to try to find the extent of infection among these birds in Chicago (5) Through the cooperation of Dr Herman N Bundesen president of the Chicago Board of Health and his staff about 200 feral pigeons were collected from various parts of Chicago

Complement fixation tests were made with blood serums of these birds against antigens prepared from lung tissue of mice infected with the psittacosis virus 6 BC and Illinois virus respectively Of 195 sera tested 89 or 45 per cent were positive against the Illinois virus and 87 or 44 per cent were positive against the psittacosis virus Only those serums giving fixation in a dilution of 1 20 or higher were considered positive

Virus isolation was then attempted on a large random sample of the same pigeons Suspensions of mixed lung and kidney tissue were inoculated into mice by the intranasal or intracerebral route or by both It was our impression that the intracerebral route was more productive but there was more difficulty with infection from bacterial contamination of the tissues

Tissues were inoculated into mice from 112 pigeons and 29 or 26 per cent yielded virus Of the 112 pigeons from which virus isolations were attempted 64 had complement fixing antibodies in a dilution of 1 20 or greater from these virus was isolated in 23 instances or 36 per cent Of 38 pigeons with negative complement fixation tests six or 16 per cent yielded virus These results coincide with similar observations by others that not all birds carrying virus give serologic evidence of infection

At this point it might be of interest to compare the results of studies made elsewhere to determine the extent of psittacosis infection in wild pigeons As previously stated Meyer found psittacosis antibodies in 20 of 30 pigeons he examined in New York City Levinson *et al* (6) examined 14 pigeons collected in Philadelphia

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Psittacosis in Wild Pigeons

THE importance of domestic pigeons as a reservoir of psittacosis infection has been well investigated Since the first mention of the presence of this disease in domestic pigeons by Cole (*1*) in 1940 there have been many references (2 3) to human cases occuring in proximity to pigeon lofts or by contact with the birds themselves In some cases the investigators have noted that the pigeons were sick or dying from being overcrowded or kept under insanitary conditions

Although the natural history of the disease in wild pigeons is not as well understood there is no dearth of evidence regarding the extent to which they harbor viruses of the psittacosis group It is not strange that wild pigeons are also susceptible to naturally occurring psittacosis since domestic pigeons are believed to be descendants of a single species of wild pigeons more properly called rock doves (*Columba livia*) Meyer (2) in 1941 seems to have recorded the first proved cases of psittacosis in wild pigeons He mentioned the circumstances surrounding the development of psittacosis in two women about two weeks after they had picked up a sick wild pigeon in the streets of New York City The period of contact was not prolonged because the pigeon died four days later Of 30 pigeons collected from streets in the same vicinity a few days later 20 had complement fixing titers against psittacosis three in high titer

of the 29 viruses isolated Groups of six mice each were immunized by injecting them with living virus subcutaneously and they were subsequently challenged with decimal dilutions of virus by the intracerebral route Control of the virulence of the virus preparation employed in the challenges was obtained by simultaneous injection of normal mice with the same decimal dilutions

Mice were first immunized with each of the six viruses isolated from pigeons (viruses numbers 30 84 146 159 220 and 224) and challenged with the following psittacosis 6 BC ornithosis 207 SF 470 meningopneumonitis MP F 97 and Illinois viruses The results are shown in Table II In this table the control data regard

TABLE II Cross immunity tests in mice immunized with unidentified pigeon viruses and challenged with viruses of the psittacosis group

Immunizing Viruses	Challenging Viruses—0 03 c c i c †				
	Psitt	*Ornith*	*SF*	*MP*	*Ill*
30	−	+	+	−	−
84	−	+	+	−	−
146	−	−	+	−	−
159	−	−	+	−	−
2 0	−	−	∓	−	+
224	−	−	∓	−	+

Numbers refer to serial numbers of pigeons from which viruses were isolated
† i c—intracerebral inoculation

ing cross immunity between the homologous viruses have been omitted for the sake of brevity in all cases the results in these control tests were those expected The reverse procedure was then followed immunizing the mice with the psittacosis ornithosis meningopneumonitis and Illinois viruses and then challenging them with the six pigeon virus strains The results of these tests are shown in Table III

Allowing for the inherent defects of these tests and remembering that the psittacosis virus strain employed was apparently more antigenic than the others used we would interpret these findings as placing strains 30 and 84 in the ornithosis group strains 146 and 159 in none of the groups and strains 220 and 224 in the same

six or 42 per cent had positive complement fixation tests Labzoffsky (7) found the incidence (based on serum titers of 1 10 or higher) in three Ontario cities to be as follows city A four of nine (45 per cent) city B one of 43 (2 per cent) and city C 19 of 94 (20 per cent) He isolated virus from 14 of the 24 (58 per cent) sero positive pigeons or from 16 per cent of all the birds studied Davis and Ewing (8) isolated virus from 15 of 100 feral pigeons collected in Baltimore In 1946–47 Davis (9) attempted virus isolation from 96 adult pigeons collected in Washington D C without success In 1949 he found three of 22 adult pigeons in the same city carrying virus whereas none of 32 squabs tested was harboring virus Five of 27 squabs had positive complement fixation tests Davis also in 1949 isolated virus from five of 120 pigeons collected in Birmingham Alabama It is of interest that 60 per cent of 91 pigeons from this same group gave positive complement fixation tests Meyer and Eddie (*10*) reported that only one of 100 pigeons they examined in Mexico showed serologic evidence of infection It may be only coincidence that there seems to be a steadily decreasing incidence of virus carriage by wild pigeons as southern latitudes are reached (Table I)

TABLE I Incidence of psittacosis virus carriage by adult pigeons in various areas in the United States Canada and Mexico

AREA	*No Pigeons Studied*	*Per cent with Positive C F*	*Per cent Virus Isolated*
New York City	50	67	—
Philadelphia	14	42	—
Chicago	195	45	26
Ontario	146	17	16
Baltimore	100	—	15
Washington D C	118	35	2
Birmingham Ala	120	60	4
Mexico	100	1	—

Identity of Chicago Pigeon Viruses

It had been planned originally to identify all of the viruses which were isolated from Chicago pigeons Because of developments which are extraneous to this discussion it was possible to attempt identification by means of cross protection tests of only six

of the 29 viruses isolated Groups of six mice each were immunized by injecting them with living virus subcutaneously and they were subsequently challenged with decimal dilutions of virus by the intracerebral route Control of the virulence of the virus preparation employed in the challenges was obtained by simultaneous injection of normal mice with the same decimal dilutions

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Numbers refer to serial numbers of pigeons from which viruses were isolated
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TABLE III Cross immunity tests in mice immu nized with viruses of the psittacosis group and challenged with unidenti fied pigeon viruses

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	30	84	146	159	220	224
Psitt	+	+	−	+	−	∓
Omith	−	+	−	−	−	−
SF	−	−	−	−	−	−
MP	−	−	−	−	−	−
Ill	−	−	−	−	∓	+

* Numbers refer to serial numbers of pigeons from which viruses were isolated
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group as the Illinois virus The latter finding is especially important because it has been apparently assumed by some that the Illinois virus since it was isolated from patients not in contact with psit tacine birds is probably of mammalian origin It is unfortunate that the results of these groupings obtained by cross immunity tests could not have been compared with the results obtained by one of the procedures not then in use such as the neutralization agglutination inhibition or toxin neutralization tests If Chicago pigeons were carrying the Illinois type of virus it is probable that they were harboring a highly fatal strain as shown by the virulence tests in animals and the fact that this strain originally came from fatal human cases of psittacosis *It is also important to record that* a microbiologist working in our laboratory became infected with the Illinois virus and his subsequent illness was severe

Pigeons as a Source of Human Infection

It may be seen that if 4 to 26 per cent of wild pigeons in this country are harboring psittacosis virus they represent a vast po tential reservoir of this virus However it must be remembered that the virus isolations were made from the internal organs of the pigeons and this does not prove that they were shedding virus One would assume that virus in the kidney probably means virus in the urine and thus in the cloacal contents however the amount and virulence of the virus actually excreted is unknown

Probably the extent of human infection from this source cannot

be ascertained with any degree of certainty Meyer and Eddie (*10*) in a summary of the sources of cases of psittacosis in the United States from 1945 to July 1950 state that pigeons alone were responsible for 69 cases and three deaths This compares with 98 cases and one death attributed to psittacine birds and 93 cases and three deaths from all other known and unknown sources during the same period Perhaps most of the cases and deaths tabulated by Meyer and Eddie were from contact with domestic rather than wild pigeons

Another way of approaching the problem is to attempt to estimate the percentage of cases of primary atypical pneumonia which are caused by viruses of the psittacosis group Smadel (*11*) estimated this at about 25 per cent Florman and Weiss (*12*) at 26 per cent Morgan and Finland (*13*) at about 5 per cent and Eaton (*14*) at under 10 per cent These estimates were based on serologic studies conducted on fairly large samples of patients with the acute disease All were made before the beginning of the present mania for keeping parakeets At the time it appeared that a reasonable explanation of these findings might be that people were being exposed to some large reservoir such as apparently exists in wild pigeons The recent discovery of reservoirs of viruses of the psittacosis lymphogranuloma venereum group in mammals such as cats and the demonstration that human carriage of psittacosis virus can occur (*15*) indicate that there may be large reservoirs of virus other than the one in pigeons

On the other hand there is reason to believe that the history of a patient with psittacosis is usually so poorly taken that his contact even with a sick pigeon several days or weeks before onset of his illness would not be detected Infection of the type that might occur in a large city i e from air borne dried pigeon droppings would probably go undetected in most instances There is reason to suspect that laboratory confirmation is rarely sought by physicians except in severe cases of respiratory infection and then only occasionally Therefore the extent of human infection with psittacosis and especially the role of the wild pigeon in disseminating it is difficult to elucidate

There is some evidence incriminating feral pigeons Meyer s two cases in contact with a sick pigeon have been mentioned Mor

TABLE III Cross immunity tests in mice immunized with viruses of the psittacosis group and challenged with unidentified pigeon viruses

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SF	−	−	−	−	−	−
MP	−	−	−	−	−	−
Ill.	−	−	−	−	∓	+

* Numbers refer to serial numbers of pigeons from which viruses were isolated

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from two of the pigeons Unfortunately it was impossible to attempt virus isolations from the men

If this outbreak was caused by wild pigeons it has several features of interest Insofar as could be determined no sick pigeons were found in the car shop prior to or during the outbreak. The explanation for the outbreak may be that among the larger number of pigeons in the building there were enough open carriers or shedders of the virus to contaminate the surroundings with unusually large amounts of virus This coupled with the fact that the shop seemed to act as a wind tunnel could have caused the men to be exposed to large amounts of virus laden dried pigeon excreta

If dried wind blown excreta are capable of infecting people one would imagine that places where pigeons congregate such as in small parks or plazas and on elevated railroad station platforms might provide ideal conditions for spreading virus on windy days or even on still days when the beating of the birds wings stir up the dried excreta Yet it must be acknowledged that except in the outbreak described above most of the recorded cases of psittacosis associated with wild pigeons seem to have been due to close contact with sick or dead pigeons

It would appear that the conditions favoring infection of man are much more likely to be present when a parakeet is kept in a small apartment in very close association with a family than when there is the ordinary type of contact with wild pigeons or their excreta Thus all except two of the 15 cases of pneumonitis coming to our attention in Illinois last year as a result of positive laboratory tests for psittacosis had a history of contact with psittacine birds A diagnosis of lymphogranuloma venereum was probable in both of these

Conclusion

It must be concluded that this is another of the animal human disease relationships in which all of the ecologic factors are not known Further studies to fill in the gaps in our knowledge are sorely needed Because of the filth wild pigeons create it may be desirable to try to eradicate them but insofar as the spread of

gan and Finland (*13*) have shown that of 89 patients with primary atypical pneumonia six had positive complement fixation tests for psittacosis Four of these had close contacts with wild pigeons three handled sick or dead pigeons Levinson *et al* (*6*) reported six cases of psittacosis which they attributed to contact with pigeons Two of these were exposed to domestic pigeons The other four had no contact with psittacine birds but lived in an area infested with large numbers of pigeons some of which rested on the window sills of the patients dwellings The pigeons were found to have serologic evidence of infection Meiklejohn *et al* (*16*) reported four cases of psittacosis from contact with pigeons one patient had fed wild pigeons from the window of her apartment the others had contact with domestic pigeons Cohen *et al* (*17*) reported that a man and his wife developed pneumonitis one week after they began keeping two pigeons (domestic or wild not indicated) in their bedroom Both had good increases in titers for psittacosis complement fixing antibodies Other sporadic cases of psittacosis occurring in individuals who fed or handled feral pigeons have been mentioned to us verbally but unfortunately have not been recorded in the literature

An outbreak in which wild pigeons were incriminated was studied by us in March 1946 At that time seven middle aged men employed in a railroad car shop in a Central Illinois city developed virus pneumonia" Investigation revealed that the men were working in a large barn like structure with many windows and doors most of which were kept open during working hours The interior was windswept and dusty Pigeons flew in and out at will and were so numerous that it was reported by the foreman that over 300 had been shot about six weeks before the outbreak began Pigeons were less numerous at the time of the investigation but it was estimated that there were at least 60 Pigeon droppings were seen on the structural elements of the building and on the steel plates which had been installed to keep the droppings out of the drinking fountains

Serums from six of the seven patients gave positive complement fixation tests for psittacosis as did the serums of five of the 13 pigeons tested A virus falling in the psittacosis group was isolated

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Psittacosis in Seashore Birds

PSITTACOSIS infection appears to have a world wide distri bution and the incidence of related viruses among birds and mam mals might warrant a statement that this family of agents is more deeply and widely embedded in nature than most other viral in fections We are still attracted by the prospect of exploring new species of birds for infection although most of those thus far studied appear to carry the virus

When Rasmussen described 174 pneumonia cases in Faroe Is landers (1) cases which were identified as psittacosis (2 3) this was acclaimed by some as an exceptional circumstance and by others as portending a broader spectrum of infection than the name implied (4) This epidemic represents the first recorded ex ample of psittacosis as an occupational disease it came from processing young fulmar petrels for food Subsequent studies by Coles (5) and by Meyer (6) in which psittacosis virus was dem onstrated in other non psittacine birds warranted the recom mendation by Meyer that this disease henceforth be called ornitho sis (7)

"Bayou fever" in Louisiana was identified as ornithotic pneu monitis but the origin of the human infections remains obscure (8 9) Psittacine species of birds were not associated with this epidemic It has been stated that virus recovered from non psit tacine birds was less virulent for humans than that from psittacine species but this has been based more on impression than experi

psittacosis is concerned there does not seem to be scientific evidence at this time that they are of sufficient importance to justify attempts to eliminate them The public however, should be warned that intimate contact with these birds especially those that are sick or dead is potentially dangerous

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Most of the serum titrations of the positive birds rarely exceeded a dilution of 1 10 although one reached 1 320

Since evidence of infection appeared to be more frequent in three species of birds (laughing gull willet and skimmer) it was felt that the virus might be obtained more readily from these Pooled liver and kidney emulsions from gulls and from willets yielded virus when inoculated intracerebrally and intraperitoneally into white mice Each pool contained tissues from two birds A total of eight pools of gull tissues and six of willet tissues were inoculated From these four viruses were isolated and all were identified as members of the lymphogranuloma venereum psittacosis group Two of the viruses were from willets and two from laughing gulls The viruses were identified by conventional criteria of morphology staining properties and serologic relationship to known viruses of this group Skimmers were not examined for virus however in view of the high incidence of positive serologic reactions among them successful virus isolations from these would not have been surprising It is possible that others of the species examined might approach the high serologic incidence here described providing that a more sensitive test were employed such as the complement fixation inhibition test

One of the willet strains isolated was infectious for mice by the intracerebral and by the intraperitoneal routes It killed mice with meningoencephalitis two to three days after intracerebral inoculation and it killed one rabbit with similar lesions on the fourth day following intracerebral inoculation (*12*)

All of the birds collected in this survey were simultaneously examined for ectoparasites These parasites were checked only for identification The three species of birds with the highest incidence of serologic reactions also had the heaviest infestations with ectoparasites Whether an insanitary environment is conducive to both ornithosis and infestation or whether the ectoparasites are responsible for some of the infection is now being studied

It is the consensus among investigators (though insufficiently documented) that the ornithosis agent is disseminated among birds through fecal contamination of food and air A recent study in our laboratory (*13*) indicates that young ducklings may manifest sustained viremia for periods well over two weeks in duration

ence The virus recovered from the bayou fever epidemic of 1944 was as virulent in animals as were any viruses isolated from psittacine birds and a more recently identified virus from a naturally infected egret was equally as virulent (*10*)

Most investigators will now agree that ornithosis is no rare infection among seashore birds Our serologic survey of seashore birds collected on Galveston Island Texas, would indicate that this infective agent resides among several of the species examined (*11*) In this survey 165 birds among 13 species were shot and immediately bled from the heart The serums were examined for complement fixing properties with washed antigen prepared from the P_4 strain of ornithosis virus in chorioallantoic fluid Table I indicates that three species of birds had infection rates higher than 40 per cent These were the laughing gull (*Larus atricilla L*)

TABLE I Ornithosis in seashore birds as shown by the complement fixation test

Species	No Tested	No AC	No +	% +	Complement fixation serum titers					
					1 5	1 10	1 20	1 40	1 80	1 3_0
Laughing gull	*60*	*3*	*24*	*40*	*10*	*8*	*1*	*2*	*2*	*1*
Willet	37	3	17	45 4	7	6	4			
Skimmer	15	0	8	53	4	2	2			
Sanderling	8	1	4	50	4					
Royal tern	15	0	1	6			1			
Least tern	8	0	2	20		1		1		
Common tern	6	0	3	50	1	2				
Gull billed tern	6	0	1	16		1				
Ring billed gull	1	0	0							
Glossy ibis	1	0	1						1	
Brown pelican	4	1	0							
Sooty tern	1	0	0							
Reddish egret	3	3	0							
	165	11	61	36	26	20	8	3	3	1

AC—anticomplementary

the willet (*Catoptrophorus semipalmatus G*) and the skimmer (*Rhynchops nigra L*) There were not sufficient numbers to warrant a percentage calculation however three other species of birds demonstrated serologic evidence of infection among them These were the sanderling (*Crocethia alba P*) the least tern (*Sterna albifrons L*) and the common tern (*Sterna hirundo L*)

Most of the serum titrations of the positive birds rarely exceeded a dilution of 1 10 although one reached 1 320

Since evidence of infection appeared to be more frequent in three species of birds (laughing gull willet and skimmer) it was felt that the virus might be obtained more readily from these Pooled liver and kidney emulsions from gulls and from willets yielded virus when inoculated intracerebrally and intraperitoneally into white mice Each pool contained tissues from two birds A total of eight pools of gull tissues and six of willet tissues were inoculated From these four viruses were isolated and all were identified as members of the lymphogranuloma venereum psitta cosis group Two of the viruses were from willets and two from laughing gulls The viruses were identified by conventional criteria of morphology staining properties and serologic relationship to known viruses of this group Skimmers were not examined for virus however in view of the high incidence of positive serologic reactions among them successful virus isolations from these would not have been surprising It is possible that others of the species examined might approach the high serologic incidence here de scribed providing that a more sensitive test were employed such as the complement fixation inhibition test

One of the willet strains isolated was infectious for mice by the intracerebral and by the intraperitoneal routes It killed mice with meningoencephalitis two to three days after intracerebral inocula tion and it killed one rabbit with similar lesions on the fourth day following intracerebral inoculation (12)

All of the birds collected in this survey were simultaneously examined for ectoparasites These parasites were checked only for identification The three species of birds with the highest inci dence of serologic reactions also had the heaviest infestations with ectoparasites Whether an insanitary environment is conducive to both ornithosis and infestation or whether the ectoparasites are responsible for some of the infection is now being studied

It is the consensus among investigators (though insufficiently documented) that the ornithosis agent is disseminated among birds through fecal contamination of food and air A recent study in our laboratory (13) indicates that young ducklings may mani fest sustained viremia for periods well over two weeks in duration.

A prolonged viremia is without doubt an important premise of insect passage and this is being studied rather intensively as a possible secondary route

Added support to the prevalence of ornithosis infection in aquatic birds comes from a subsequent report (*14*) of virus isolation from herring gulls (*Larus argentatus*) and of serologic evidence of this disease in the lesser black gull (*L fuscus*) As already stated the virus has also been demonstrated by Rubin *et al* in the blood of a naturally infected snowy egret (*Leucophoyx thula*) (*15*) (See Tables II and III)

TABLE II Isolation of ornithosis virus from seashore birds by investigator and year

SPECIES	INVESTIGATOR
Fulmar petrel (*Fulmarus glacialis*)	Haagen and Maurer 1938
Sea gulls	Meyer and Eddie 1945
Laughing gull (*Larus atricilla*)	Pollard 1947
Willet (*Catoptrophorus semipalmatus*)	Pollard 1947
Herring gull (*Larus argentatus*)	Miles and Shrivastan 1951
Snowy egret (*Leucophoyx thula*)	Rubin *et al* 1951

TABLE III Serologic evidence of ornithosis in seashore birds other than those from which virus was isolated

SPECIES	INVESTIGATOR
Skimmer (*Rhynchops nigra L*)	Pollard 1947
Sanderling (*Crocethia alba*)	
Least tern (*Sterna albifrons*)	" "
Common tern (*Sterna hirundo L*)	"
Lesser black gull (*L fuscus*)	Miles and Shrivastan 1951

Accurate serologic surveys for retrospective evidence of ornithosis infections among humans residing in the Gulf Coast area will be difficult or impossible because of the relatively high prevalence of lymphogranuloma venereum infection The latter infection is not uncommon among Negroes and is also encountered among Latin American groups It is not yet possible to differentiate lymphogranuloma venereum and ornithosis infections solely by complement fixation and skin test reactions Only the rise in antibody titer coincident with recovery from a nonvenereal infection or isolation of virus from sputum might serve to support a diagnosis of ornithosis

Conclusions

In retrospect it is not surprising that seashore birds are infected with ornithosis The surprising thing is that more humans are not infected from such sources Of course if man should contrive to live as intimately with seashore birds as he does with the more domesticated species then the wild birds might constitute a serious health hazard This has been clearly exemplified with the serious outbreak of ornithotic pneumonia in the Faroe Islands (*1*) The importance of ornithosis in species of seashore birds aside from its importance as a hazard to human health is a potential or real threat to the health of our domesticated birds Thus far evidence would indicate that the latter problem is inapparent or absent Ornithosis infections have been associated with sick chickens (*7*) but the implication of spread from seashore birds is not even suggested as yet

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9 Olson B J and Larson C L An epidemic of a severe pneumonitis in the bayou region of Louisiana V Etiology *Pub Health Rep.,* 60 1488–503 (1945)

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LEONARD J GOSS, D V M , PH D

12

Psittacosis in Zoological Collections

ALTHOUGH psittacosis is commonly associated with parrot and parrot like (psittacine) birds few cases have been diagnosed in birds on exhibition in zoos in the United States In fact only one such outbreak has been reported in the literature (1) That was in the National Zoo in Washington D C in 1941 In this outbreak two birdhouse keepers who became ill showed symptoms sugges tive of psittacosis and their illness was so diagnosed clinically Virus recovery from the human patients was not attempted due to ab sence of sputum samples blood tests apparently were not con ducted Both patients survived The first of the two had been em ployed as a keeper for 33 days The second was one of seven other employees who had worked as keepers in the birdhouse from three to 20 years

Between January 21 1941 and April 7 1941 60 birds from the National Zoo collection were examined 51 had died and nine were destroyed Of these 15 were positive (12 psittacines and three non psittacines—one Java sparrow two African doves) Forty three birds were negative and two questionable but probably negative Among the negatives and questionable the distribution was as shown on page 106

No mention is made in this report as to which of the birds died or were destroyed The total number of birds in the psittacine collec tion at the time was slightly over 100 This zoo has averaged about 100 psittacines in its collection at all times for over 60 years Since 1937 all psittacines entering that collection had been quarantined

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per zoo Something over 4 000 psittacines had been exhibited by the 27 zoos since their beginning

The annual public attendance at these zoos is over 25 000 000

Mortality in the psittacine collections is low the zoo exhibiting 281 birds reported that seven to ten die each year Seven of the 27 zoos do not have their birds autopsied Only two of the 27 had ever had psittacosis diagnosed in their collections (New York and Washington D C)

In 1941 the New York Zoological Society began conducting complement fixation tests on the psittacines in its Bronx Zoo bird collection This was prompted by the finding of gross lesions suggestive of psittacosis in a dead bird unfortunately the bird was immediately autoclaved At that time the collection consisted of approximately 100 psittacines A test and slaughter system was instituted Each bird was bled from the wing vein and serum submitted to the George Williams Hooper Foundation for complement fixation testing Each bird that gave a positive reaction in the complement fixation test was destroyed and submitted for virus recovery attempts For 19 months during 1941 and 1942 over 400 serum samples were tested from the 100 birds Forty three birds were destroyed because of positive tests and an additional 20 died Of the 63 birds that died or were destroyed (all of which were submitted for virus recovery attempts) only two were positive One of these the laboratory reported as "a mild latent infection" and the other showed microscopic lesions only and was considered "not an actual spreader"

During the testing procedure a bleeding technique was developed Even the smallest lovebirds were successfully bled. The first bird bled was not anesthetized and it fractured a humerus in thrashing about Following this each bird was given ether A pledget of cotton soaked with ether was placed over the nostrils and beak while the bird was held lying flat on a table with its wings snugly held against its body Anesthesia was produced by intermittently removing the ether soaked cotton to permit the inhalation of fresh air When anesthesia reached the level where the bird was relaxed *one wing was extended and blood taken from the radial* vein by means of a hypodermic needle and syringe 23° or 25° needles were needed for the smaller birds The syringe contained a

Negative		*Questionable*	
Psittacines	8	Psittacine	1
Java sparrow	1	Pigeon	1
Doves	2		
Eagle	2		
Gull	1		
Duck	3		
Pheasant	2		
Stork	1		
Owl	2		
Cowbird	1		
Flycatcher	1		
Quail	2		
Pigeon	1		
Finch	16		

for three weeks All birds that died during the quarantine period were examined for psittacosis by the National Institute of Health None of the 39 examined was positive

In the summer of 1951 the Columbus Ohio Zoo imported African Gray parrots from South Africa and macaws from South America These birds were isolated" some died and from one the virus of psittacosis was recovered Simultaneously the curator of birds became ill and a diagnosis of psittacosis was made This was based on the clinical picture and a positive complement fixation test (1 128)

The Tracy Aviary Salt Lake City in the past 15 years has imported a considerable number of psittacines with no losses from psittacosis Of 146 birds imported four losses occurred in transit This same aviary lost in transit or destroyed on arrival 38 of 51 birds from Singapore This shipment was overcrowded and poorly crated The remainder of the lot survived a six months quarantine

The Philadelphia Zoo reports that between 1901 and 1952 it has autopsied 60 Loridae and 1 514 Psittacidae without a single bird showing lesions suggestive of psittacosis

In 1950 the American Veterinary Medical Association Advisory Committee on Diseases of Wild Animals surveyed the larger principal zoological parks in the United States and Canada Twenty seven replied to the questionnaire sent out Three of the 27 were located in Canada A summary of the results follows

These zoos had exhibited psittacines for as long as 75 years the average being 30 years Some of them exhibit as many as 281 birds at a time The total exhibited in 1950 was 1 497 or an average of 76

in contact with birds from 4 to 20 years were sent to San Francisco Antibody tests were negative on three and positive to a titer of 4 on the fourth

The San Diego Zoo which has served as detention quarters for thousands of birds since the repeal of the Interstate Transportation Law has encountered psittacosis only once In the winter of 1952–53 a customs agent brought a large confiscated shipment to the San Diego Zoo during very adverse weather conditions The agent became ill and was treated by a Navy hospital for simple pneumonia which two weeks after hospitalization was diagnosed (method not known) as psittacosis The disease was then diagnosed by Dr K F Meyer in some of the birds in the shipment The entire shipment was then destroyed with the exception of 100 that had already gone to another zoo Of the 100 shipped elsewhere none died At the time of the quarantine of the lot that proved to contain positive birds there were about 3 700 birds in the same quarantine quarters

Java rice birds have always been kept with the psittacines at the San Diego Zoo No known cases of psittacosis have occurred in them

In the thousands of birds quarantined at the San Diego Zoo there has not been a single case of psittacosis in the personnel caring for the birds Years ago several dozen birds were destroyed and sampled from the San Diego Zoo collection (which has always numbered approximately 300) and not a single case of psittacosis was laboratory diagnosed

In performing autopsies on approximately 200 psittacines in the past 14 years only twice have I seen gross lesions suggestive of psittacosis The first one probably was negative but was not checked for virus The second one proved positive It was reported by Drachman (2) The bird was an African Gray parrot the only bird owned by a physician for over 20 years Three years prior to the bird s death it was owned by a dentist and his family of six On the same premises were ducks geese and chickens About April 12 1952 two parakeets from a New York City pet shop were introduced into the household On May 8 the mother developed chills cough and fever the father became ill on May 15 and a thirteen year old daughter on May 18 The mother s illness persisted for three months and the father s for four months All three human patients gave

death There was *no spread* from nonfatal cases, not even mild ill nesses were found among their contacts Similarly there was no spread to those who were in contact with fatal cases only in the early stages of the disease

This infectiousness of fatal cases late in the course of the disease apparently was not related to the simple presence of the etiologic agent in the upper respiratory tract The virus was demonstrated in

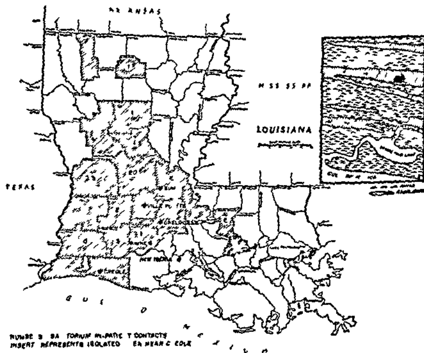


Figure 1 Area of study in the 1943 epidemic of Louisiana pneumonitis Reprinted from Olson and Treuting, *Public Health Reports* 59 1299–1311 (1944) by permission.

the throat washings of a nonfatal case (no 16) on the ninth day of illness and in a fatal case (no 17) on the third day of a ten day total illness (4) Perhaps the amount of virus increased in the sputum in late phases of fatal cases or perhaps the virus became more adapted and hence more infectious for man Nor was the infectiousness of fatal cases related to the degree of exposure All of

the secondary cases were in individuals who experienced a heavy exposure but not necessarily a prolonged one On the other hand a number of people were heavily exposed and did not contract the disease

The conclusion was reached that transmission of the disease was through direct contact with a previous case probably respiratory No psittacine birds were present in any of the homes or in the sanatorium there was no common food or water supply no evidence could be found of an intermediate insect vector

Possible Reservoirs of Infection

The origin of the epidemic was not discovered although the possibility of an animal reservoir bird or mammal was recognized because of the nature of the region in which the first case lived During the investigation it was discovered that a somewhat similar epidemic occurred in February and March 1936 in Rayne Louisiana a community east of the site of the first case in the 1943 epidemic In this episode there were eight cases with six deaths It cannot be proved that this was an epidemic of the same disease but, as described by the attending physicians and members of the families involved it had a certain similarity clinically and epidemiologically

As previously mentioned a virus demonstrated to be a member of the psittacosis ornithosis group was isolated from throat washings of patients It was also isolated from the blood of one patient and from autopsy specimens of lung and spleen (1) The virus has been differentiated from psittacosis virus and meningopneumonitis virus by its effect on laboratory animals (5)

The possibility even probability that there exists a bird reservoir of the virus of Louisiana pneumonitis cannot be disregarded Recent findings of investigators at the Virus and Rickettsial Section of the Communicable Disease Center of the United States Public Health Service strengthen this In June 1950 a virus was isolated from the blood of two nestling snowy egrets from the Gulf coast of southeastern Louisiana The virus was identified as a member of the psittacosis-ornithosis group resembling closely the Louisiana pneumonitis virus This was an adventitious isolation made during investigations to determine animal reservoirs of Eastern equine encephalomyelitis (6) The same investigators found that a virus

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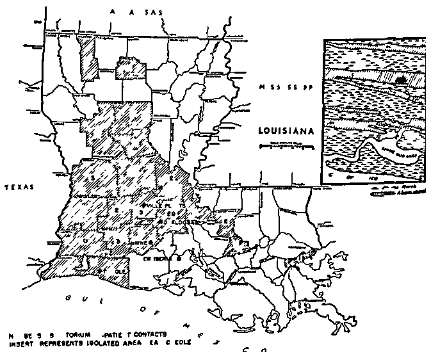


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originated there It is considered that the epizootic was introduced because one or more of the birds was harboring a subclinical infection with the disease upon arrival at the laboratory (7)

Conclusions

No human cases of Louisiana pneumonitis have been reported from the area since 1943 The potentiality of recurrence exists however It can be reasonably concluded that a reservoir exists in nature and that the virus isolated therefrom is a virulent one Because of the habits and customs of many of the residents of the area the risk of exposure exists One may speculate that the disease does occur but is unrecognized since it cannot be differentiated from other viral pneumonias by clinical examination alone Or it may normally occur in a mild nonfatal form that would not raise the suspicion of those in attendance If the latter is the case it becomes necessary to speculate further that some change in the host parasite relationship must occur to produce an epidemic such as that of 1943 It is thus apparent that a number of important questions remain to be answered with regard to the occurrence of this disease in humans in the area The virus isolations from wild egrets are of considerable public health importance and should be a stimulus to further study

To close on a more comforting note one may point out that both the original Louisiana pneumonitis strain of virus and the strains recently isolated from egrets have proved in experimental infections to be very susceptible to the new antibiotics Hence should an outbreak of this disease be initiated in man probably effective means of treatment (and prevention of spread from man to man) would be at hand

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identical in all its characteristics to that isolated in 1950 was the cause of a highly fatal epizootic in wild American and snowy egrets captured in southeastern Louisiana The nineteen young American egrets and 23 young snowy egrets involved in this epizootic were captured and brought to the laboratory in June 1951, the epizootic occurred during August and ensuing months of that year Conditions in the laboratory were such that it is unlikely that the epizootic

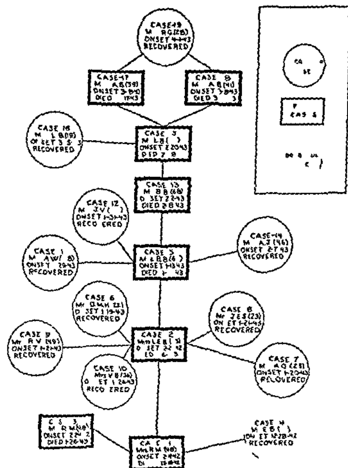


Figure 2 Spread of the disease in the 1943 epidemic of Louisiana pneumonitis Reprinted from Olson and Treuting, *Public Health Reports* 59 1299–1311 (1944) by permission

L L PARKS, M D

14 Report on the Psittacosis Problem in Florida

THE question of whether or not psittacosis is a public health problem of any importance has been before us for many years in Florida In checking with our Bureau of Preventable Diseases on the number of human cases of psittacosis recorded in Florida we find that only one human case has been reported in the past 12 years This case was reported in Miami Beach but the health officer was never positive that it was actually a case of psittacosis as there was not sufficient supporting evidence However it was recorded clinically as such

Up until 1951 the Florida State Board of Health had regulations on the shipment of psittacine birds On March 1 1951 our bureau suggested to the state health officer that inasmuch as there had been so much confusion on the control of psittacosis and since the interstate shipment of birds was creating an unsatisfactory relationship with many of the bird dealers in the state the issuing of permits be discontinued We recommended that the bird dealers comply with the requirements of the United States Public Health Service

Prior to the making of this recommendation to the state health officer we were being called upon to inspect the various bird farms We were asked to approve or disapprove these places of business There were no particular standards set when we inspected these bird farms In general the standards were that the farm should observe ordinary sanitation and should have satisfactory isolation facilities We did not require that a record be kept on where the

4 Olson B J and Larson C L An epidemic of a severe pneumonitis in the bayou region of Louisiana V Etiology *Pub Health Rep* 60 1488–1503 (1945)

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fornia papers carried headlines with the largest letters available just as we do in Florida when we hear of the earthquakes or en cephalomyelitis problems in California On February 13 1952 we received a copy of a letter from the Minnesota State Health Depart ment stating that a physician s twenty year old son had returned from Florida with a bird which he had purchased from a Florida pet shop The bird sickened several days after arrival died and conse quently was destroyed The boy s mother developed an illness as did his father also and both came down with symptoms that ap peared to be psittacosis Complement fixation tests were reported positive in a dilution high enough to confirm the diagnosis of psit tacosis This seemed to be our first positive information that there had been any human cases and as it later developed as far as I know these are the only cases that appeared from February 1952 to 1954 that could possibly be blamed on the State of Florida

At this point we sought the advice and assistance of the Com municable Disease Center (CDC) in Atlanta The services of vet erinarians physicians and virologists were supplied to Florida All of these workers came in and did a splendid job of investigating in their respective fields The material for this paper is based primarily upon the findings of these individual workers

When the positive diagnosis of psittacosis was made there was a question as to what control measures should be instituted It was the opinion of some individuals that a state quarantine should be declared and that no birds should be moved from the State of Flor ida Our office was not particularly alarmed over the positive find ings of psittacosis in the state and actually discouraged any state quarantine because it was believed by us that as long as there were no human cases there was no real public health problem It was the general belief of a number of our local health officers that this was simply a condition which probably had existed among the birds for many years To enforce such a quarantine would require all of the National Guard of Florida and then it probably could not be en forced effectively It was realized that birds are transported across the state line not only by private automobiles but by planes trains and every other means of transportation The requiring of permits for the shipment of birds across the state line is not practical It is unfair to have such restrictions for only the honest people would

birds came from or to whom they were sold We realized that the bird business in Florida is a very large one there are an estimated 6 000 bird dealers or more in the City of Miami and surrounding Dade County We also realized that many conscientious bird deal ers were trying to comply with the regulations even though some times their competitors were not so conscientious The regulations if they may be called such were making it hard on some of the bird dealers at the same time many of them were not being supervised at all Sufficient staff to supervise all of these dealers properly not just in the Miami area but throughout the state was not available Inasmuch as no human cases of *psittacosis* had been reported it was felt that we should repeal all our regulations in the control of psittacine birds This matter was presented to the State Board of Health in regular session in July 1951 all regulations pertaining to the control of psittacine birds were repealed

Then on January 7 1952 a parrot which had died in Broward County Fort Lauderdale was suspected of having had psittacosis This dead bird was shipped to the National Institute of Health and on January 15 1952 it was reported that the bird had the psit tacosis virus This bird had been on display at the Beach Inn in Pompano Beach Florida The county health officer of that county quarantined the premises and took serologic specimens from the human contacts he further advised the inn operator that the inn might re open as soon as the premises had been thoroughly cleaned and all exposed birds placed in special quarantine cages and re moved from the premises One of the macaws which was also in this display later proved to be positive for the psittacosis virus It was learned that the birds in the display all came from a bird farm in South Florida that had had other losses in its parakeets This farm was located in an adjacent county and a ban was placed on the selling of the birds from this place It was learned that the first bird along with other birds that had died had been purchased during October and November of 1951 by a bird dealer across the state in Tampa

As soon as the cause of death in the first bird was diagnosed as psittacosis the news began popping out all over the country with the implication that we in Florida were trying to hide our problems in order to keep up the tourist trade I can imagine that the Cali

have caused human illness not merely in Minnesota Connecticut and Indiana but also in Florida No procedure except the isolation of the virus from a patient will provide such a proof

It was our opinion at the time that no quarantine measures were necessary until more evidence was produced that we actually had a public health problem We were most willing to have all the investigations made that could be made and the following plan of procedure was agreed upon by all those concerned

1 History and paired serums were obtained from every person exposed to known psittacosis in birds As the list of infected premises grew other people were included

2 Attempts were made to trace infected birds to the aviary of their origin With the failure to trace specific birds to specific premises all probable sources were canvassed

3 Such other sources and sales outlets of psittaciformes as came to attention were visited also to seek evidence of bird or human morbidity suggestive of psittacosis and to obtain appropriate human and bird specimens

4 Health department and various hospital records on recent cases of pulmonary and influenza like infection were reviewed From these were selected a group of follow up patients who were canvassed from the standpoint of exposure to psittacine and other birds prior to onset of their illness Serums were obtained from those whose history was relevant

5 News releases and educational materials were disseminated from the county health departments as well as the state board of health urging people who owned sold or raised parakeets to be alert to the condition of their birds and to the appearance of influenza like symptoms Veterinarians treating sick birds were cautioned to seek laboratory diagnosis of suspected or suspicious birds before ruling out psittacosis Practicing physicians were cautioned to be cognizant of psittacine or other avian contact of all patients with acute illnesses and to obtain diagnostic specimens if such contact was suspected

To help get this information on to the veterinarians and physicians of the state memorandums were sent to all of the county health departments and medical society secretaries These memorandums explained to the physicians the type of specimens to take

comply with them and many would not comply at all through ignorance of the restrictions

Our bureau was very happy to have the assistance of the many staff members from CDC that were made available to help us find out if we did or did not have a real problem A number of conferences were held with the members of the CDC staff We had the assistance of Dr K F Meyer of the University of California and *it is interesting to note that in a letter from him* dated March 27 1952 to one of the CDC investigators he states he feels that this condition in Florida was simply a reactivation due to the greed of the bird dealers and particularly the breeders Dr Meyer found in 1941 that the disease was prevalent in one of the pet shops in Miami It is also interesting to note from this correspondence from Dr Meyers that he states "Surely the type of surveillance you will give the aviaries will give you the same picture that we found in California in the early thirties You must sample the aviaries by taking ten per cent of the birds and testing their organs on mice This procedure and this alone will give you a true picture of the extent of the infection any other is incomplete

"Human infections will doubtless come to light Many are probably subclinical and may antedate the present period of great interest This aspect I have discussed repeatedly The present parakeet strains are probably not very toxic"

Dr Meyer went ahead to explain the interpretation of the complement fixation test with the psittacosis antigen He stated that a titer of 1 16 or less is of no diagnostic significance A titer of 1 32 is a "suspect and a titer of 1 64 makes a specific infection very probable A titer of 1 128 or over makes it almost certain

He further advised that it is necessary to repeat the titration test and that a rise in titer in two subsequent samples should be at least fourfold to be of diagnostic value He states further that the diagnosis of psittacosis should not be made unless the above mentioned serologic changes are found and unless the clinical and epidemiologic facts make such a diagnosis probable

A part of this same letter of information of recent date from Dr Meyer is quoted as follows

If I appraise the situation correctly you wish to establish that the proven parrot and parakeet infections in the aviaries of Florida

TABLE II Relation of the presence of antibodies against psittacosis to previous respiratory illness among poultry dressing workers in Florida

Serologic Category	*Previous Respiratory Illness*	*No History of Illness*	*Total*
Psittacosis positive—Syphilis positive	4	6	10
Psittacosis positive—Syphilis negative	1	4	5
Psittacosis negative—Syphilis positive	0	2	2
Psittacosis negative—Syphilis negative	1	8	9
TOTAL	6	20	26

A second blood test was obtained from those poultry workers who had a psittacosis titer of 1 16 and over The results of this second test were reported but it was impossible to determine whether these results reflect previous contact with psittacosis virus or cross reactions with syphilis or lymphogranuloma venereum To quote from Dr Herbert G Stoenner s report "At the time this survey among poultry processing plant employees was contemplated it was not expected that syphilis would be so prevalent among this group as food handler s health certificates are required for employment In order to bring all their employee health cards up to date the manager of the first plant surveyed requested that the blood specimens be tested also for syphilis Of 26 serums collected in this plant 12 showed serologic evidence of syphilis Ten of the 12 were positive also for psittacosis whereas only five of 14 non syphilitic serums were positive for psittacosis This evidence suggests that most of the positive complement fixation tests for psittacosis are the result of the reaction of the yolk sac material in the psittacosis antigen with syphilitic serum "

Of the total laboratory examinations made of various psittacine birds the following is a summary of the examinations made These specimens were collected by the different investigators and represent the over all picture

A total of 105 parakeets were examined for psittacosis virus and 14 or 13 3 per cent were positive Sixty nine pigeons were examined and none was positive of four pigeon serums examined one was positive with a titer of 1 32 and the others were positive in a dilution of less than 1 8 One macaw was examined and was

and how to submit them for examination (One physician submitted specimens of what he thought was psittacosis but they proved to be an influenza virus) News articles asking the physicians to be on the lookout for this disease were sent to the state medical journals and to two of the larger counties that have a medical society bulletin

Along with the other control methods efforts were made to have the bird shops and farms improve their general sanitation and also to provide isolation facilities for the birds that became ill

One of the problems first noticed in making the study was that some bird dealers were notorious for not remembering where their birds were purchased and to whom they sold them This made it most difficult to trace down the source of origin of most stocks of birds

If we were to get a true picture of our conditions it seemed advisable to take samples of birds not only in the Miami and Fort Lauderdale areas but also in other parts of the state It was further believed that we should take samples of the air in bird houses from which psittacosis virus had previously been isolated It was decided that we should study some of the poultry slaughter houses to see if we could obtain any information pertaining not only to the poultry, but to the workers in these slaughter houses as well Two hundred chicken spleens were obtained from broilers processed in the poultry plants and the results of these examinations were all negative

In correlating the presence of antibodies against psittacosis with a history of previous respiratory illness among poultry dressing workers the following data were found

TABLE I Relation of previou respiratory illness to the presence of antibodies against psittacosis among poultry dressing workers in Florida

SEROLOGIC CATEGORY	*Previous Respiratory Illness*	*No History of Illness*	*Total*
Psittacosis positive 1 8 or greater	10	19	29
Psittacosis negative 1 8 or less	3	21	24
TOTAL	13	40	53

standing and cooperation there can be among county state and federal health services What might have turned out to be a period of chaos caused by undue and undeserving newspaper publicity was kept within reason If an experience of this nature should occur at some time in the future in our state we should again welcome the assistance which we had during this period from the United States Public Health Service There was in every way a most satisfactory working relationship between agencies concerned

The ban on the importation of psittacine birds into the State of Florida from foreign countries (which was due to the diagnosis of psittacosis in birds in some of our aviaries) was lifted in July 1952 We still use the foreign quarantine regulations and all foreign bird imports are quarantined Quarantine facilities of the United States Public Health Service and the state are used

The developments since this paper was originally prepared have not changed except that in March 1953 we received word that a person in Pennsylvania had developed psittacosis and that this patient had recently purchased a parakeet from a bird dealer in Daytona Beach This bird dealer when visited stated that he had had no sick birds Inasmuch as there was some question in mind as to the condition of his birds his place of business was quarantined for a period of 14 days Bird specimens were submitted to the Virus Laboratory in Montgomery Alabama even though at the time of the visit of our veterinarian there was no evidence of illness among the birds

When I was in Miami I discussed the question of psittacosis with Dr T E Cato the county health officer and he feels as most of us do that undoubtedly the pigeons chickens sea gulls and probably many of the other birds are most likely carriers of this disease To limit our control measures to psittacine birds alone will have little or no effect on the control of psittacosis in humans

Summary

This is a report of an outbreak of psittacosis in birds in Florida in which 14 parakeets out of 105 examined were found to be positive There were no human cases reported in the state however there were two cases reported in the State of Minnesota in patients who had a bird recently purchased in Florida This bird was not ex

positive Other birds including one Cock O the Rock one canary one toucan and six others which were not specified were also examined but all were negative

Of the human serums examined 94 were positive in a dilution of less than 1 8 and three were positive in a dilution of 1 16 One examination was repeated in this 1 16 group and the second test was the same as the first Two spinal fluids were examined with the result that psittacosis was positive in a dilution of less than 1 8 Seven sputums were examined and none was positive

The bird droppings from eight chicken farms were examined and psittacosis was reported negative Twenty chicken spleens were examined and all were negative 12 were specimens from the non virus infected farms where examinations were all reported as negative Thirty three workers in a poultry house in Jacksonville were examined by blood test and all were negative

There were eight bird farms or pet shops in which a positive diagnosis of psittacosis was established

All of the virus examinations were done by the United States Public Health Service Virus Laboratory Montgomery Alabama

In the follow up of bird handlers poultry workers and the follow up of hospital patients who had a virus infection there were no human cases of psittacosis diagnosed during the entire period

Conclusions

Psittacosis in our opinion is a questionable minor public health problem in the State of Florida It may be that this disease is of greater importance in some of the northern states than it is in Florida for the reason that pet birds in Florida are not confined so closely to human habitation as they are in the North There is a freer circulation of air and of course more sunshine in Florida and perhaps the virus is less toxic in the South Also with the newer antibiotics the treatment of psittacosis seems to be reasonably satisfactory The bird industry in the State of Florida is a rather large one and it is a difficult industry to regulate because of our rapid means of communication Until there is more evidence I believe that this experience has shown that psittacosis is of minor importance as a public health problem in this state To me this experience in the psittacosis problem is a good example of what good under

CALVIN B SPENCER, M D

15

Development of United States Foreign Quarantine Regulations for the Control of Psittacosis Since 1930

ALTHOUGH psittacosis had been known for many years as a disease affecting birds of the parrot family with occasional transmission to man it was not until the pandemic of 1929 and 1930 when it occurred as a severe disease affecting man that its significance as a public health problem was recognized From November 23 1929 to December 31 1930 170 cases of psittacosis with 33 deaths were reported in the United States Parrots were the principal vectors being involved in the development of at least 55 of the 74 foci of infection (*1 2*) As a result an Executive Order (No 5264) was issued on January 24 1930 which placed a temporary embargo on importation of parrots and in effect placed responsibility upon the Surgeon General of the United States Public Health Service to make regulations to control their importation and to prevent further introduction of psittacosis into the United States

It would be inappropriate at this time to quote in full the regulations and amendments promulgated under this Executive Order but in view of the continuing interest in the psittacosis problem a brief review of our pertinent foreign quarantine controls may help point up the general problem

Regulations, 1930–1932

On February 3 1930 the Surgeon General issued regulations strictly limiting the importation of parrots These regulations did

amined but the complement fixation tests of the two patients were sufficiently high to make the diagnosis of psittacosis positive

The use of permits in the control of this disease is not a practical control procedure with the present rapid means of transportation

This experience is one in which the United States Public Health Service Communicable Disease Center in Atlanta made well trained workers available to assist us There was cooperation on the part of all concerned to make the study as worthwhile as possible

It is apparent from this study that psittacosis is probably endemic among parakeets and most likely among other birds Control measures are very difficult with our present knowledge of the disease

African grays cockatoos macaws lories parakeets lovebirds and all similar birds"

These regulations added the requirement that shipments of more than five birds were to be accompanied by a certificate from the health authority at place of origin to the effect that the birds had been inspected and found apparently well and in good sanitary condition also that the aviary or other establishment of origin was maintained in good sanitary condition and apparently free of psittacosis infection

A little more than a year later on December 20 1933 the regulations were again revised in an attempt to afford more protection against importation of the infection Psittacine birds under eight months of age were excluded from admission to the country because it had been found that psittacosis was more likely to be transmitted by young birds (5) The certification as to the place of origin of imported birds was to be supplemented by such laboratory examination of birds as the certifying health authority deemed necessary to enable him to determine that the birds to be shipped were free of psittacosis The quarantine officer at the port of arrival was authorized to select birds for laboratory examination before releasing a shipment from detention Also prior to a bird s release the state health officer having jurisdiction at its final destination was to be notified of the action

Regulations, 1939–1946

These regulations were not substantially revised again for more than five years until 1939 During that period experience had shown that psittacosis was not controlled and that numerous human cases were still occurring moreover because of improved laboratory methods of isolation of the etiologic agent the carrier state was known to go on indefinitely without symptoms or detection The regulations of May 3 1939 required a United States Public Health Service permit for importation of psittacine birds for commercial purposes for zoological exhibits or for scientific study The death of birds en route to this country was to be reported to the quarantine officer at the port of arrival The quarantine period at the port of arrival was extended to six months for commercial shipments From each commercial shipment of 100 or more birds 10

not pertain to other psittacine birds such as cockatoos macaws and parakeets (3) An individual privately owned parrot appearing to be well could be brought in by its owner if it was to be transported directly to the home of the owner, and if it was certified that the bird had been maintained in a good sanitary environment in the owner s quarters for not less than 60 days prior to arrival and that it had not been exposed meanwhile to contact with other parrots

On October 29 1930 the regulations were revised to permit importation of parrots in shipments not exceeding 100 birds under sanitary restrictions relating to crating feeding watering and protection en route especially protection from extreme cold A 15-day quarantine period was required at the port of arrival this detention period was to be extended for such time as the Surgeon General deemed necessary when suspicious illness was observed among the birds Importation of these large shipments was limited to ports at which federal quarantine detention facilities were maintained Whenever practicable concurrence of an inspector of the Department of Agriculture s Biological Survey was to be obtained before shipments were released from quarantine

Individual privately owned parrots not exceeding five in number could be brought in by the owner without detention subject to the same requirements specified by the earlier regulation

For the purpose of the revised regulations the term parrots was extended to include all species commonly known by that name such as Amazons African grays cockatoos and lories Parakeets lovebirds and other small psittacine birds could be brought in without quarantine detention if transported in approved sanitary crates containing not more than 25 birds each and providing not less than 0 5 cu ft of space per bird

Regulations, 1932–1939

The regulations were revised October 6 1932 applying import restrictions equally to all psittacine birds It had been determined meanwhile that shell parakeets probably because of their greater numbers in commerce were more significant vectors of psittacosis than parrots and that most if not all psittacine birds were actual or potential vectors (4) The revised regulations referred to "all birds commonly known as parrots Amazons Mexican double heads

African grays cockatoos macaws lories parakeets lovebirds and all similar birds"

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per cent were to be submitted for laboratory examination from shipments of fewer than 100 birds ten were to be submitted for such examination unless the shipment consisted of fewer than 20 birds in which case half of them were to be taken for examination If the sample birds were found infected all other birds in the shipment were to be destroyed Birds that became ill during quarantine detention were to undergo laboratory examination and if these were found infected, all birds in the shipment were to be destroyed.

Birds imported for exhibition at zoological gardens or parks or for scientific study at established research institutions could undergo detention and isolation at destination rather than at the port quarantine station

An individual could now bring in a maximum of three pet birds not subject to detention if they were found healthy by a United States Public Health Service medical officer if the owner certified that they had been in his possession for the preceding two years without contact with similar birds and if they would be taken immediately to his residence and not offered for sale barter or as gifts or for public exhibition

These regulations of 1939 discontinued the requirements regarding crating specifications and sanitation en route

Regulations, 1946–1951

Probably the most significant change in regulations was made on July 1 1946 As of that date commercial shipments of psittacine birds were no longer permitted since many states or cities prohibited introduction of psittacines and since cases of psittacosis continued to occur Importation of birds for other purposes remained substantially the same except that sanitary certificates of origin were abolished An individual was permitted to bring in only two pet birds instead of three as formerly and was required to certify that he had owned them for two years and kept them in his own quarters with no contact with other psittacine birds

Reappraisal of Regulations in 1951

In 1951 a board of officers appointed by the Surgeon General to reappraise the quarantine regulations for psittacosis control was instructed to report on the following subjects (1) current con

cepts of the epidemiology of psittacosis (2) a brief appraisal of therapeutic agents (3) recommended changes in the foreign quar antine regulations (4) recommended changes in the interstate quarantine regulations (5) methods for coordinating national and interstate control measures with local health department con trol measures and (6) other recommendations on the subject deemed pertinent by the board

The report of the board submitted June 1 1951 stated that for eign quarantine control should be continued for a number of rea sons

1 The history of the world wide outbreaks of psittacosis in man in the 1929-1930 period revealed that the most important source of infection was imported psittacine birds

2 The last large commercial shipments offered for importation into the United States and submitted to study revealed that the ma jority of birds were infected with psittacosis (*1*)

3 Since the institution of regulations prohibiting the commer cial importation of psittacine birds there had been no large out breaks of psittacosis in man comparable to those of 1929-1930

4 Competent authorities believed that the unlimited importa tion of psittacine birds would increase the psittacosis reservoir that was in close contact with man especially that maintained by the large psittacine birds which rarely breed in temperate zones

5 It was impossible to determine psittacosis free areas abroad from which birds could be imported Also if disease were traced to imported birds it would be difficult to determine their original source to prevent further shipments from that area

On December 15 1951 the regulations were revised to incorpo rate changes recommended by the Surgeon Generals board and certain other amendments The principal changes were as follows

1 With approval of the Surgeon General birds could be im ported for "medical research" Formerly the corresponding pro vision had referred to importation by a research institution"

2 Those persons applying for a permit to import birds for a zo ological park were required to provide suitable facilities to isolate the birds from human beings and from other birds until certain no psittacosis was present It was stated that the birds must appear healthy to the quarantine officer at the port of entry and must be

per cent were to be submitted for laboratory examination from shipments of fewer than 100 birds ten were to be submitted for such examination unless the shipment consisted of fewer than 20 birds in which case half of them were to be taken for examination If the sample birds were found infected all other birds in the ship ment were to be destroyed Birds that became ill during quarantine detention were to undergo laboratory examination and if these were found infected, all birds in the shipment were to be destroyed

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Reappraisal of Regulations in 1951

In 1951 a board of officers appointed by the Surgeon General to reappraise the quarantine regulations for psittacosis control was instructed to report on the following subjects (1) current con

of infection and adopt measures calculated to minimize or eliminate it

However when imported birds are introduced into collections of domestically raised birds not only is the pool of potential psittacosis infection directly increased but the probability of transmission of psittacosis from imported birds to domestic birds is added Also variant strains of psittacosis virus may in this manner be introduced among domestic birds—which may be much more susceptible to these strains than to those already occurring among birds in this country Thus the sale of birds from flocks in which domestic and imported birds are intermingled may present a greater hazard of infection than sales from flocks restricted to domestic birds Hence it was deemed advisable to provide by regulation that an establishment which claims to be a zoological park but which engages in the business of selling psittacine birds either imported or domestic to dealers or to the public may not be permitted to import psittacine birds

Other changes incorporated in the present regulations include the following (1) The limitation on importation of pet birds has been changed to two birds per family instead of per person (2) A United States Public Health Service permit may be obtained for the return of psittacine birds to the United States when more than two birds are taken out of the country or when birds taken out of the country have been in the owner s possession for less than four months at the time of return (3) All psittacine birds showing symptoms suggestive of psittacosis shall be destroyed immediately at the port of arrival (4) Psittacine birds shall be excluded if during shipment they have been in contact with birds showing symptoms suggestive of psittacosis or with birds dying from unconfirmed causes

When a zoological park applies for a permit to import psittacine birds the United States Public Health Service must determine whether the park has proper facilities for quarantining the birds for at least 30 days In the majority of cases veterinarians or other qualified personnel in the appropriate state or local health departments are asked for recommendations on the approval of the detention facilities Establishments are not approved if they fail to meet state and local health requirements and copies of all import permits are sent to the appropriate state health departments

isolated for at least 30 days on arrival at the park Any birds in isolation displaying symptoms suggestive of psittacosis were to be killed and submitted for laboratory examination it was required that a report of the results be made to the Surgeon General

3 It was no longer specified that birds must be eight months or more of age

4 Birds brought in as pets were no longer required to be accompanied by the owner and transported to his residence The period of possession before importation was reduced from two years to four months The owner was to attest that the birds were not intended for sale in the United States and that he had not imported any other pet psittacine birds in the past 12 months

Regulations of 1953

The latest revision of the regulations was on February 21 1953 It was necessary to modify provisions regarding disposal of birds denied entry in order to avoid any implication that the government would re establish detention facilities for birds

The other principal change was the definition of zoological park" for purposes of the import regulations The term "zoological park" is now limited to a place or an establishment that exhibits birds or other animals but does not sell or trade psittacine birds to the public or to dealers in birds

In order to reduce the danger of introduction and spread of psittacosis in the United States it has been found necessary to place a restriction upon the categories of individuals who may bring in psittacine birds and in some cases upon the number of birds that may be brought in This restriction has a twofold purpose First by limiting the number of birds that may be imported the potential pool of psittacosis infection is correspondingly restricted Second by prohibiting importation of the birds for commercial purposes the danger of widespread continuous exposure of individuals to the risk of psittacosis infection from imported birds is minimized

The provision of the regulations which authorize the granting of permits to import psittacine birds destined to zoological parks recognizes that although there may be a danger of infection from birds exhibited in zoological parks individuals are not continuously exposed to such a risk Further such parks are alert to the danger

could have been the source of introduction of disease into other bird groups including poultry The Commissioner of Customs has reported that illegal international traffic in psittacine birds presented a major customs enforcement problem in 1952 A customs report from San Diego stated that in one month—December 1952—officials seized 2 960 birds 1 440 were taken in one seizure Customs agents have obtained evidence indicating that large numbers of the birds have recently been brought into Mexico from Europe intended for the profitable United States market The potential of these activities has been estimated at a quarter of a million dollars annually

The conditions of crowding and undernourishment common with respect to smuggled birds are favorable to the development of active infection in birds carrying the virus Late in 1952 a customs agent who had assisted in the seizure of birds smuggled from Mexico suffered a prolonged illness a diagnosis of psittacosis was confirmed by laboratory tests Dr K F Meyer diagnosed psittacosis in birds taken from the lot handled by this customs official and in numerous other sample birds taken from among more than 3 000 which had been seized at the Mexican border during November and December 1952

Since the outbreaks of 1929–1930 the incidence of human psittacosis in this country has been at a relatively low level However the need for controlling the disease continues to confront us and this control has become largely an economic and industrial problem especially in recent years In 1952 for instance of the 135 cases reported to the United States Public Health Service 61 are known to have occurred among employees of packing plants in Texas where turkeys were being processed

In view of what we know of the history of psittacosis with its tendency to spread by affecting other species including mammals we might well take warning that uncontrolled importation of animal life without due regard to its significance from a public health standpoint may bring severe problems either in the health or the industrial field We shall expect the researchers to go apace in assisting us to understand the control problems involved

Effective quarantine control is dependent upon collaboration

In general, the following standards are applied in determining whether a zoological park has adequate quarantine facilities

1 The facilities should be in a separate building used only for quarantine purposes Persons other than those tending the birds should not be permitted to come within 50 feet of the building Quarantine signs should be posted prohibiting unauthorized persons from entering the quarantine area

2 The facilities should be of impermeable construction with smooth surfaces that can be readily cleaned and disinfected

3 Openings should be no larger than necessary for ventilation so located and protected as to minimize the possibility that the psittacosis virus may pass to the outside

4 The facilities should be equipped with a sanitary waste disposal system

With regard to pet birds some difficulty has been encountered with the interpretation of the word possession" in the regulatory provision that the birds must have been in the owner s possession for the four months preceding arrival The Division of Foreign Quarantine has recently suggested to the Department of State that it furnish the following instructions to United States consulates, for the guidance of persons inquiring about bringing pet psittacine birds to this country In the owner s possession for the four months preceding arrival" is to be interpeted as follows The owner must (a) have had the birds with him for the entire four months (except for arrival on separate conveyances), or (b) have had a place of abode where he kept the birds and where he spent sufficient time to give reasonable assurance of having firsthand knowledge of the condition of the birds during the four months"

Problems of Control

Although the Division of Foreign Quarantine has applied a variety of controls to the importation of psittacine birds it is recognized that these measures have not been fully effective However, there have been comparatively few reports of psittacosis introduced by birds imported in accordance with United States Public Health Service regulations We must consider that many psittacine birds have been brought in illegally and some of these

could have been the source of introduction of disease into other bird groups including poultry The Commissioner of Customs has reported that illegal international traffic in psittacine birds presented a major customs enforcement problem in 1952 A customs report from San Diego stated that in one month—December 1952—officials seized 2 960 birds 1 440 were taken in one seizure Customs agents have obtained evidence indicating that large numbers of the birds have recently been brought into Mexico from Europe intended for the profitable United States market The potential of these activities has been estimated at a quarter of a million dollars annually

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Effective quarantine control is dependent upon collaboration

from several directions—upon cooperation among countries and after importation has occurred cooperation among federal state and local authorities within the nation

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HERALD R COX, SC D

16 Chemotherapy of Psittacosis

THE principal clinical manifestation of psittacosis is a pneumonitis characterized by headache malaise a dry nonproductive cough chills and as a rule physical signs of pneumonia The leucocyte count rarely exceeds 12 000 cells per cu mm of blood and X ray of the chest during the acute phase usually shows signs of pulmonary involvement The duration of fever in 17 sporadic cases studied by Davis (1) ranged from six to 60 days with the median case having 17 days of fever Diagnosis may be made by isolation of the virus from the sputum or body tissues or by use of the complement fixation test wherein a fourfold rise in titer during the course of the disease is considered to be diagnostically significant Primary atypical pneumonia and Q fever both resemble psittacosis and can be differentiated with certainty only by laboratory procedures

During the last few years drug therapy has been found to be particularly successful against the infections caused by rickettsias and by the large viral agents comprising the psittacosis lymphogranuloma venereum group

Para aminobenzoic Acid

Hamilton (2) demonstrated that para aminobenzoic acid had no inhibiting effect on the growth of psittacosis virus in developing chick embryos when the drug was administered at the time of infection

Acridines

Hurst (*3*) reported that Nitroakridin 3582 (Hochst) possessed moderate therapeutic activity against the viruses of psittacosis and lymphogranuloma venereum Nitroakridin was more active in mice than either sulfadiazine or sulfamezathine but was less active than penicillin However a week or more after completion of a prolonged course of either Nitroakridin or penicillin mice which apparently had withstood infection with psittacosis frequently developed symptoms and died The virus was not eliminated by drug treatment but persisted and was able to multiply in a fully virulent state

Eaton van Allen and Wiener (*4*) reported acriflavine 3 nitro 6 7-dimethoxy 9 (2 phenyl-4-diethylaminobutylamino) acridine and 3-nitro-6 7 dimethoxy 9 (2 hydroxy-3 diethylaminopropyl amino) acridine inhibited the growth of feline pneumonitis lymphogranuloma venereum and meningopneumonitis viruses in the yolk sac of the chick embryo Proflavine atabrine and compounds closely related to the above-named nitroacridines except for substitution of Cl for NO_2 had no significant inhibitory action 3 nitro-9-aminoacridine was found to be intermediate in its effect

Sulfonamides

Many of the psittacosis virus strains have been found to be in sensitive to sulfonamides either in mice (*5 6*) or in man(*7 8 9*) and these agents are now considered to be of little therapeutic value in the disease However under experimental conditions in tissue cultures mice and chick embryos the 6 BC strain of psittacosis a parakeet strain has been shown to be susceptible to sulfadiazine (*10 11*) sulfathiazole and sulfamerazine (*12*) as well as to penicillin but not to para–aminobenzoic acid or streptomycin (*10*) By repeated passage of the 6 BC strain in the presence of sulfadiazine a strain of virus was developed which was completely resistant to 20 mg of the drug even after ten passages through normal chick embryos Similar resistance of the strain was induced also against sulfathiazole and sulfamerazine (*12*) The fact that both para aminobenzoic acid and pteroylglutamic acid are actively antagonistic against large doses of sulfadiazine suggests that the

primary action of sulfadiazine on the 6 BC strain of psittacosis is against the incorporation of para aminobenzoic acid into pteroyl glutamic acid (*13*)

Penicillin Laboratory Data

Parker and Diefendorf (*14*) noted a definite inhibiting or retarding effect of penicillin on the multiplication of the 6 BC strain of psittacosis in tissue culture and in the developing chick embryo Not all embryos that died were tested for the presence of virus but sufficient work was done to show that in a fair number of embryos that died late after penicillin treatment, no virus could be detected in their tissues

Heilman and Herrell (*15 16*) were the first to show that experimental psittacosis in the mouse responds to penicillin The amount of penicillin used was very large 1 000 units per mouse divided into five doses over 24 hours and repeated for seven to 12 days But the results obtained were quite significant particularly in the second paper in which all of 52 untreated control mice died whereas of 52 treated mice only four died giving a mortality rate of 8 per cent However virus was not eliminated from the tissues of the treated mice since it was recovered from the livers and spleens of 11 of 12 mice tested

Bedson and May (*17*) confirmed the findings of Heilman and Herrell (*15 16*) but noted that the quantity of penicillin required to keep the infection subclinical in mice is very great and that a comparable dosage for a man would be approximately 11 million units of penicillin per person

Penicillin Clinical Results

Therapeutic effectiveness of penicillin treatment of humans with psittacosis has been reported by Turgasen (*18*) Flippin Gaydosh and Fittipoldi (*19*) Parker (*20*) Kirkwood (*21*) Meyer and Eddie (*22*) Rosebury Ellingson Meiklejohn and Schabel (*23*) Wolins (*24*) Goggio (*25*) Lawrence and Johnston (*26*) and Tasker (*27*) It should be noted however that the dosage of penicillin employed in most of these cases was very much less than that which would be considered necessary on the basis of the laboratory experimental work reported particularly by Heilman and Herrell (*16*) and Bed

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on the thirteenth day of illness She became afebrile by the sixteenth day Complement fixation tests showed titers of 1 16 on the twelfth day of illness and 1 64 on the twenty third day

Kirkwood (*21*) reported a woman aged fifty two who had received adequate doses of sulfadiazine during the early part of illness without effect Penicillin was started on the tenth day of illness at which time the fever was 105 F giving an initial dose of 20 000 units followed by 10 000 units every three hours until 500 000 units were given Within 48 hours the temperature dropped to 99 5 – 101 F and within 96 hours the temperature was normal and remained so The patient s sense of well being improved as soon as penicillin therapy was started Complement fixation tests carried out by Dr Meyer on a blood sample taken on the eighteenth day of illness showed titers of 4+ 1 128 and 3+ 1 256

Meyer and Eddie (*22*) have reported penicillin treatment under careful supervision of three patients who acquired laboratory infections Virus was demonstrated in the blood of all three and in the sputum of one In one case penicillin therapy was started on the sixth day of the patient s illness using an initial dose of 80 000 units followed during the next 24 hours with 160 000 units Treatment with 160 000 units per day was maintained for six days followed by 110 000 units for one day and 80 000 units for two days The patient became afebrile and remained so following the initial dose A second patient who was more severely infected was started on penicillin therapy on the tenth day of illness The amount of penicillin given was 220 000 units on the first day 320 000 units on the second day and gradually decreasing amounts thereafter for ten days The patient showed clinical improvement starting 48 hours after penicillin treatment These authors point out that serious relapses have in some instances resulted from the inadequate treatment of the disease They strongly urged that a high dosage level be administered in rapid succession early in the course of the infection so that the viral elements will be suppressed as completely as possible and thus prevent the future development of virus carriers

Rosebury *et al* (*23*) reported a case of a laboratory worker who became infected with the 6 BC strain of psittacosis which was shown by Early and Morgan (*10 11*) and Golub (*12*) to be sensi

son and May (*17*) Furthermore in most of the cases, penicillin therapy was not initiated until quite late in the course of the disease

Turgasen (*18*) reported a patient aged forty three a pigeon fancier who received a daily dosage of 100 000 units of penicillin intramuscularly in split doses for seven and a half days starting on the fifth day of illness The response was not dramatic since the high febrile condition persisted for five days after start of treatment Penicillin was stopped when the temperature had been normal approximately three days The patient apparently was fully recovered one month after onset of illness A complement fixation test carried out by Dr Karl F Meyer on a blood sample taken the fifth day of illness showed a titer of 1 256

Flippin Gaydosh and Fittipoldi (*19*) reported a woman aged fifty two who was referred to the hospital on the ninth day of illness because of a pneumonia that did not yield to sulfonamide therapy On the four days prior to hospitalization she had received a total of 12 gm sulfadiazine Sulfamerazine was used in place of sulfadiazine but since it produced no effect it was stopped on the nineteenth day Intramuscular penicillin in doses of 100 000 units daily was initiated on the nineteenth day and was continued for six days The patient showed marked clinical improvement within 36 hours after starting penicillin therapy It should be noted however, that penicillin was given quite late in the course of the disease when spontaneous recovery might have been expected to occur so that recovery in this case cannot be definitely attributed to treatment with penicillin No attempt was made to isolate virus at any time but a blood sample sent to Dr Meyer on the twenty third day of illness showed a complement fixation titer of 1 128

Parker (*20*) reported on two patients The first a sixty one year old woman was ill two weeks before being hospitalized Sodium sulfadiazine was given for three days with no apparent effect Intramuscular penicillin in a dosage of 120 000 units per day was started on the nineteenth day Clinical improvement was seen on the twenty first day Complement fixation tests performed by Dr Meyer showed a titer of 1 128 on the twenty first day and a titer of 1 256 during convalescence The second patient a fifty two-year old woman was started on penicillin therapy, 160 000 units per day

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tive to both penicillin and sulfadiazine Prior to infection the patient had received three injections of formalinized psittacosis vaccine prepared from infected yolk sac tissues The patient a man aged forty-one was treated with both penicillin and sulfadiazine 400 000 units of penicillin were given daily from the fourth to fourteenth days of illness and 6 gm of sulfadiazine were given daily from the fifth to twelfth days The patient became afebrile and showed marked clinical improvement as early as the sixth day of illness after he had received only 800 000 units of penicillin and 4 gm of sulfadiazine The diagnosis of psittacosis was confirmed by isolation of the virus from the sputum and a rise of complement fixing antibody in the blood The virus isolated was found to be sensitive to both penicillin and sulfadiazine therapy experimentally in mice

Wolins (21) reported on eight cases of ornithosis or psittacosis in man resulting from contact with infected domestic Pekin ducks Two patients were treated with sulfadiazine three were treated with sulfadiazine and penicillin and three were treated with penicillin alone Of the two sulfadiazine treated patients one was a very mild case that responded promptly to small doses of the drug The second case showed no apparent improvement on 1 gm sulfadiazine every four hours for four days The drug was discontinued and the patient continued to show fever for five more days after which defervescence and recovery took place Of the three patients who received both sulfadiazine and penicillin therapy the first was given sulfadiazine in doses of 1 gm every four hours for two days with no response Sulfadiazine was then discontinued, and penicillin in doses of 25 000 units every three hours was given for two days Because no clinical improvement was noted the penicillin dosage was increased to 50 000 units every three hours There was then a prompt response with temperature falling to normal within 24 hours The second patient was started promptly on penicillin using 400 000 units daily There was no response in five days of treatment Sulfadiazine was then started with an initial dose of 3 gm and subsequent doses of 1 gm every four hours The temperature returned to normal within 48 hours The third patient likewise showed no improvement on four days of penicillin treat

ment 400 000 units per day but responded rapidly when given sulfadiazine 1 gm every four hours Of the three patients who received penicillin therapy alone two responded well with return of temperature to normal within 48 to 72 hours using 50 000 units of penicillin every three hours The third patient who was the only one of the eight who could be considered to be critically ill failed to improve on penicillin dosages of 25 000 units every four or three hours but did finally improve on 50 000 units every three hours

Goggio (25) has reported a case in which penicillin therapy was started reasonably early in the course of the disease The dosage given was quite large and the response appeared to be good The patient a twenty seven year old woman was given a first injection of 300 000 units of penicillin intramuscularly followed by 100 000 units every three hours Since improvement was not evident at the end of 24 hours the dosage was increased to 200 000 units every three hours and maintained at that level for five days The patient began to improve rapidly by the third day and was normal by the fifth day Virus was recovered from the patient s sputum about six days after onset of disease and approximately 24 hours after 300 000 units of penicillin had been given

Lawrence and Johnston (26) reported a case in a fifty year-old colored male who had been given sulfadiazine with no improvement in his condition Penicillin therapy in doses of 300 000 units every three hours was started on approximately the fourteenth day of illness Within 24 hours the patient was completely afebrile and presented marked clinical improvement

Tasker (27) reported a case in a medical research worker aged forty three who contracted the disease by handling infected mice Penicillin therapy was started on the seventh day of illness giving 1,200 000 units in three doses the first day 1 500 000 units in two doses the second day and 1 000 000 units twice daily for an additional four and a half days The drug was discontinued on the sixth day after a total dosage of 11 700 000 units The response was quite gratifying since the patient became afebrile after three days of therapy

Parodi (28) has also reported on the beneficial effect of penicillin therapy

Chloromycetin (Chloramphenicol) Laboratory Data

Smadel and Jackson (29) found chloramphenicol to possess about the same degree of activity as that displayed by penicillin and sulfadiazine The drug was not virucidal *in vitro* but in mice it exerted a beneficial therapeutic effect if the mice were injected intraperitoneally but not intracerebrally The drug was found to be of value even though a period of delay took place between infection and treatment In chick embryos the drug was more effective against lymphogranuloma venereum than against psittacosis

Eaton Huang and Levenson (30) demonstrated chemotherapeutic activity against psittacosis on the part of several substituted nitrobenzenes and nitrofurans Chloramphenicol was among the compounds tested and while it was the least toxic it did not show striking superiority over other nitro compounds tested In general its activity was no greater than that of penicillin or the sulfonamides

Chloromycetin Clinical Results

Fagin and Mandiberg (31) reported a case of psittacosis in a woman aged forty nine who was a diabetic Penicillin therapy was initiated in a daily dosage of 600 000 units on the fifth day of illness but since the patient showed no improvement after being given 2 200 000 units of penicillin over a 60 hour period chloromycetin was used in addition to penicillin Three grams of chloromycetin were given as the initial dose followed by 0 5 gm every four hours The patient showed marked improvement starting 48 hours after beginning chloramphenicol therapy The dosage of penicillin was cut to 200 000 units twice a day on the fifth day of treatment and the dosage of chloramphenicol was cut to 2 gm every 24 hours on the sixth day Both drugs were discontinued on the seventh day after the patient had received a total of 5 000 000 units of penicillin and 15 gm of chloramphenicol Nausea and vomiting which were present during the period of chloramphenicol therapy subsided promptly A complement fixation test on a thirtieth day blood sample was positive in 1 128 dilution

Chapman (32) reported a case of psittacosis in a woman aged sixty one who was given 900 000 units of penicillin daily for four

days starting on the fourth day of infection without any apparent benefit Chloramphenicol therapy was started on the thirteenth day of illness giving 2 gm the first day and 1 5 gm daily for four suc cessive days The temperature dropped from 103 F to normal within 24 hours after starting chloramphenicol therapy Convales cence was quite prolonged so that another course of chloramphenı col therapy (1 5 gm per day for four days) was given two months later (August 13 to 16) The patient did not regain normal health until sometime in October Complement fixation tests made on blood samples collected on the twelfth and seventeenth days of illness were weakly positive in titers of 1 8 and 1 6 respectively The chief feature of this case was the prolonged convalescent period despite the fact that fever promptly disappeared following the first treatment with chloromycetin

Ellenbogan and Miller (33) reported a case in a sixteen year old boy who was given 1 gm of sulphatriad" every six hours for three days (a total of 11 gm) without any apparent benefit Chloram phenicol (1 gm three times per day) was then given for approxi mately three days (a total of 11 gm) The patient s temperature returned to normal within 24 hours after starting on the antibiotic

The above results obtained in clinical usage support the data ob tained in laboratory tests and show that chloramphenicol is of value in treating psittacosis in man

Aureomycin

Practically all experimental and clinical experience to date shows that aureomycin is superior to either penicillin or chloromycetin for the treatment of psittacosis infections

Aureomycin Experimental Data

Cox and Wong (34) showed that aureomycin possesses marked therapeutic activity against the viruses of the psittacosis lympho-granuloma venereum group in developing chick embryos and mice The drug was not active *in vitro* but was active *in vivo* Further more it was quite active orally as well as parenterally for mice in fected intraperitoneally or intracerebrally with thousands of lethal doses of psittacosis or lymphogranuloma venereum viruses Thus mice infected intraperitoneally with 47 000 LD_{50} of psittacosis virus

were fully protected by treating them subcutaneously with 1 mg of aureomycin per day for three successive days starting treatment 24 hours after infection Similarly mice infected intracerebrally with 13,000 LD_{50} of lymphogranuloma venereum virus were protected by treating them subcutaneously with 1 mg of aureomycin daily for seven consecutive days starting treatment 24 hours after infection However mice that were infected with large doses of psittacosis or lymphogranuloma venereum viruses and that survived as a result of treatment with aureomycin were shown to harbor the viruses in their liver and brain when sacrificed on the seventeenth day post infection

Wagner s work (35) essentially confirmed the findings of Wong and Cox (34) He tested the effects of aureomycin in both chick embryos and mice that were infected with ten different strains of virus of the psittacosis lymphogranuloma venereum group High dilutions of virus were almost completely masked by the drug as shown by survival of embryos and low titer of virus on subinocula tion As the concentration of virus increased the masking effect of the drug was shown only by survival of treated embryos not by the titer of virus that could be recovered All five virus strains tested in mice were susceptible to the antibiotic (Borg SF Gleason 6 BC and P 207 strains) Virus was recovered after 21 days from the brains of treated mice that survived

Wells and Finland (36) found that both aureomycin and chlo ramphenicol prolonged the life of chick embryos infected with the 6 BC strain of psittacosis They also found a direct relationship to exist between the dose of antibiotic given and the prolongation of life of chick embryos when the infecting virus dose was kept con stant On a weight basis aureomycin was at least three times as effective as chloramphenicol in protecting chick embryos against the dose of virus used (85 000 LD_{50}) and on a molecular basis it was five times as effective

Hurst Peters and Melvin (6) compared the therapeutic activity of penicillin chloramphenicol aureomycin and terramycin against psittacosis in the mouse and lymphogranuloma venereum virus in the mouse and chick embryo Both terramycin and aureomycin were highly active with terramycin being slightly the better Chlo- ramphenicol was third and penicillin fourth in order of activity

In mice infected intraperitoneally aureomycin and terramycin were both highly active massive doses of procaine penicillin less active and chloramphenicol only slightly active A combination of aureomycin and procaine penicillin gave results distinctly inferior to those obtained with aureomycin alone or penicillin alone This latter observation may be especially noteworthy to keep in mind when treating human cases of the disease Aureomycin was equally active whether the virus was given intraperitoneally intranasally or intracerebrally whereas procaine penicillin gave its best results only following intraperitoneal inoculation Many animals that re covered as the result of 12 or 18 days treatment and that appeared to be normal were shown to carry active virus in the spleen when sacrificed on the thirty fifth to fiftieth days

Manire and Meyer (37) tested the effects of penicillin and aureo mycin in preventing the deaths of mice due to toxin and due to in fection following the intravenous inoculation of eight different psit tacosis virus strains The toxicity of the more lethal strains (Louisi ana SF and feline pneumonitis strains) was not affected by treat ment with either aureomycin or penicillin However treatment with these drugs protected mice against death from infection with all eight viral strains Those viral strains which did not show a time lapse between toxic and infectious deaths were affected by the drugs as shown by reduced LD_{50} titers obtained in drug treated mice

Quan Meyer and Eddie (38) attempted to eliminate the all important carrier stage in infected birds by treating a series of ma ture and immature parakeets which had been infected with the Se attle strain of psittacosis of parakeet origin with aureomycin or penicillin Aqueous penicillin was given intramuscularly to one group of parakeets in a dosage of 1 000 units per bird twice a day for ten days Freshly prepared aureomycin solution was given to a sec ond group of parakeets in a dosage of 0 4 mg per bird twice a day for ten days On the twenty fifth to twenty ninth days after drug therapy was stopped all birds were sacrificed and tested for pres ence of virus in their tissues Seventy one per cent of the untreated parakeets were found to harbor virus as compared with 50 and 40 per cent of the penicillin and aureomycin treated birds respec tively Thus there was no significant reduction of the carrier rate

among the treated birds This finding of course offers little encour agement to the hope of eliminating avian carriers of psittacosis through the use of antibiotics

Wagner Andrew and Watson (39) reported that a schedule us ing intermittent aureomycin therapy gave better results in treating psittacosis infected mice than did a schedule using continuous daily therapy Similar superior results with intermittent therapy were obtained also by my former associate Dr Sam C Wong (40), in his experimental work with aureomycin in mice infected with psit tacosis and lymphogranuloma venereum viruses and scrub typhus

The intermittent type of therapy was used to good advantage by Smadel (41) and his associates in treating and preventing recurring scrub typhus infections in man with chloramphenicol

It is the author s opinion that the intermittent form of therapy is a more logical procedure to follow and should be used more often in the treatment of man particularly in the treatment of those in fections where relapses are most likely to occur—such as psittacosis scrub typhus and brucellosis

Aureomycin Clinical Results

Brainerd and his associates (42) reported three cases of psittaco sis in man in which all three patients showed a relatively poor re sponse to repository penicillin therapy but subsequently a good response to aureomycin The dosage and schedule of aureomycin therapy were not given

Lawrence and Johnston (26) reported a patient who was placed on aureomycin therapy after a combination of penicillin and sul fadiazine therapy had failed Aureomycin was started on the sixth day of hospitalization after the patient had already received 400 000 units of penicillin per day for six days and an unrecorded amount of sulfadiazine daily The initial dose of aureomycin was 0 5 gm followed by 1 0 gm in divided doses over the next six hours and then maintained at 0 25 gm dosage every four hours At the same time the dosage of penicillin was increased to 100 000 units every two hours The patient s condition improved strikingly within 36 hours after the initiation of aureomycin and increased penicillin therapy Aureomycin was discontinued on the twelfth hospital day after a total dosage of 8 5 gm was given Blood samples taken on

the twenty eighth and fifty third days both showed complement fixation titers of 1 64

Meiklejohn (*43*) refers to three patients with psittacosis who were treated with aureomycin with good results The dosage of *aureomycin used on these cases is not recorded*

Woodward (*44*) reported on a single patient with ornithosis (psittacosis) who made a dramatic response on aureomycin therapy The patient was treated on the seventh day of illness with approximately 13 gm of aureomycin being given over an eight day period 2 gm the first day 3 gm the second 2 gm the third and fourth days and 1 gm thereafter for four additional days Response to treatment took place within 24 hours Complement fixation antibodies specific for psittacosis were found in blood samples taken on the ninth seventeenth and thirty sixth days

Davis and Hawkins (*45*) reported a case of psittacosis in a fifty three year old male who was a pharmacologist at the National Institute of Health This patient was started on aureomycin therapy 80 hours after admission to the hospital A total of 14 gm of aureomycin was given in five days divided into 0 5 gm every three hours for the first 24 hours and 0 25 gm every three hours thereafter for the next four days The temperature started to decline immediately after institution of therapy and became normal and stayed that way by the thirtieth hour A blood sample taken on admission was negative whereas a sample taken on the twenty seventh day showed a positive complement fixation titer of 1 256

Attempts to isolate virus from the sputum on the third and eleventh days of illness failed. The total duration of fever was five days which is very short for a human case of psittacosis

Hamke and Russe (*46*) reported a small epidemic of psittacosis that occurred in Dortmund Germany which involved eight cases One patient was mild and seven were moderate to severely ill One patient died on the eleventh day of illness Various drugs such as Eleudron Supronal penicillin quinine calcium and streptomycin were used in three of the more severe cases without effect Aureomycin was then obtained and used in these three cases Each patient received a total dosage of 4 gm using 0 5 gm orally every four hours In the first case therapy was started on the eleventh day of illness in the second case on the twenty second day and in the

third case on the fifth day In all three patients the temperature became normal within 24 hours Aside from slight nausea no untoward reactions were encountered in using aureomycin

Green (47) has reported a case in which the patient contracted the 6 BC strain of virus by opening a broken vial of lyophilized material Aureomycin therapy was started on the second day after admission to the hospital giving 0 75 gm every six hours The patient showed no significant change in temperature during the first 24 hours of treatment but there was a reduction in the severity of his headache On the second day the headache had disappeared and there was a drop in temperature which became normal on the third day The patient was discharged from the hospital on the twelfth day after onset He had received a total dosage of 25 gm of aureomycin over a ten day period Psittacosis virus was not isolated from the blood or sputum Blood samples taken on the fourth and eighth days of illness were negative Blood samples taken on the twelfth fifteenth and seventeenth days showed complement fixation titers of 1 40 1 640 and 1 640 respectively

Ward and Birge (48) reported a case of psittacosis that occurred in the owner of a pheasant farm whose flock subsequently was found to be infected with psittacosis The authors state that a review of the literature showed no previously reported cases of psittacosis in which pheasants were involved The patient was placed on chloromycetin therapy giving 500 mg every three hours for the first six doses and 500 mg every six hours thereafter for the next seven days Due to the fact that the patient s condition remained essentially unchanged chloromycetin was discontinued and aureomycin and penicillin were substituted starting on the eighth day The dosage of the latter two antibiotics was not stated The patient gradually improved so that after ten days of combined aureomycin and penicillin therapy treatment was discontinued and the patient was considered to be recovered A blood sample taken on the fifteenth day after hospital admission showed a positive complement fixation titer of 1 64

Terramycin

Bassett (49) reported a case of psittacosis in a sixty seven year old woman who was treated with terramycin 500 mg every six

hours but the length of treatment and total dosage of drug used were not given Apparently however the results obtained were considered to be good since the author reported treating two other patients similar to the first except that they remained ambulatory The results were good All three patients showed positive complement fixation tests with psittacosis diagnostic antigen

The above cases were the only ones found in the literature that were treated with terramycin

Summary

In conclusion it is apparent that the new wide spectrum antibiotics aureomycin and terramycin give much better results both experimentally and clinically in treating psittacosis infections than do the sulfonamides penicillin or chloramphenicol Compared with aureomycin relatively few reported cases have been treated with terramycin but judging from the experimental data there is reason to believe that terramycin will give satisfactory results

In view of the great quantity of clinical data that has been accumulated with the use of aureomycin it is believed advisable to recommend that the dosage of aureomycin used for psittacosis and related viral infections be the same as that used for Rocky Mountain spotted fever typhus and other rickettsial infections (50)

It is recommended that the daily amount of aureomycin given orally be approximately 100 mg per ten pounds or 1 gm per 100 pounds body weight The total amount of drug given should be divided and administered at two to four hour intervals In order to alleviate any nausea that might occur it is best to administer each dose of antibiotic with a glassful (240 ml) of a bland drink such as milk or one ounce of milk (30 ml) to each 30 mg of aureomycin

Furthermore it must be emphasized again that none of the newer antibiotics such as aureomycin or terramycin is rickettsiocidal or virucidal in its action and accordingly may not sterilize or free the tissues of infected hosts of the agent against which it is active The antibiotics act by suppressing the growth of rickettsias or virus like organisms and permit the protective mechanism of the host to develop a state of immunity Recovery of the host in these cases depends on the development of specific immunity in which

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state the host in many instances may continue to harbor living microorganisms for a considerable period of time (*51*)

From the work of Wagner Andrew and Watson (*39*), Wong (*40*) and Smadel and his associates (*41*) the author is of the *opinion that the intermittent or interrupted noncontinuous form* of antibiotic therapy offers a much better chance of sterilizing the tissues and thereby reducing the carrier rate among infected hosts than does the use of continuous daily therapy It would be most interesting and worth while to repeat the work of Quan Meyer and Eddie (*38*) to determine if the intermittent or interrupted type of therapy might give better results than the above investigators obtained with continuous therapy in trying to eliminate the carrier state in infected avian hosts

In the case of human infections it is suggested that intermittent therapy be used as follows Administration of the antibiotic for three to five consecutive days then repeated administration of the *drug again after an interval of five to seven days This cycle of drug* therapy for three to five days followed by an interval of omission for five to seven days could be repeated two or three times if necessary to achieve the desired results

Finally attention should be drawn to the experimental findings of Hurst Peters and Melvin (*6*) who reported that a therapeutic combination of aureomycin and penicillin gave results distinctly inferior to those obtained with aureomycin alone or with penicillin alone These findings certainly indicate an antagonistic action between these two antibiotics and strongly suggest that they should not be employed simultaneously

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PROCEEDINGS OF THE SYMPOSIUM ON PSITTACOSIS

Sponsored by the New Jersey Agricultural Experiment Station Rutgers University the State University of New Jersey

With the cooperation of the Hartz Mountain Products Company

NEW YORK CITY MAY 5TH AND 6TH 1953

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Morning Session, First Day

Dr Richard E Shope of the Rockefeller Institute for Medical Research in New York presiding

CHAIRMAN SHOPE Gentlemen we are gathered for two days to discuss the problem of psittacosis

DR MARTIN Mr Chairman Gentlemen I agree with Dr Shope that we should like to see more of these conferences Those of you who work for the taxpayers will appreciate that without help we just could not have this sort of a meeting and so we are greatly indebted to the Hartz Mountain Products Company for their generosity in making this possible I know that these will be two productive days

In a sense this conference grew out of the fact that we at the New Jersey Agricultural Experiment Station have always followed the policy of working closely with industrial and commercial con cerns About 15 years ago Mr Gustav Stern of the Hartz Mountain Products Company came to us with the problem of an outbreak of pox in canaries in his Denville establishment By means of serial passage of the virus in eggs Dr F R Beaudette was able to modify the virus so that it could be used as a vaccine for the disease As a result of this and other experiences we like to think that Mr Stern developed an appreciation of the scientific approach and that this meeting is an expression of that appreciation

CHAIRMAN SHOPE Dr C E van Rooyen of the Connaught Medical Research Laboratories and the School of Hygiene Univer sity of Toronto will now present a paper "The Early History of Psittacosis"

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CHAIRMAN SHOPE Thank you Dr van Rooyen Dr W I B Beveridge of the Department of Animal Pathology University of Cambridge Cambridge England will discuss Dr van Rooyen s paper

DR BEVERIDGE I am very glad to be here and to have this

Dr Beveridge What is the distribution of psittacosis in the wild birds of Australia?

DR BEVERIDGE According to Burnet s work in 1935 there was an appreciable incidence of psittacosis in several of the species he investigated Something like 10 to 25 per cent of the birds he examined were infected No disease was noticed in the wilds at that time Burnet did find however that there was no infection in the rosellas he examined Rosellas are not gregarious they occur in groups of two or three or perhaps half a dozen and it may be due to this that the disease is not endemic in them

Then a few years later there was an outbreak of a fatal disease of parrots in Australia particularly affecting these rosellas in the southeastern part of Australia Victoria and Tasmania Burnet then found that the rosellas had psittacosis Presumably a susceptible population of rosellas had built up which then became infected from the other breeds The mortality was not confined to rosellas however That was the one instance when an epidemic was noticed in wild birds

DR STEELE Has it ever spilled over into the domestic fowl in Australia?

DR BEVERIDGE It has never been recognized

CHAIRMAN SHOPE Dr Francis B Gordon Chief of the Virus and Rickettsial Division Chemical Corps Biological Laboratories Camp Detrick Maryland will now present a paper on 'The Psittacosis Viruses A General Survey

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CHAIRMAN SHOPE Dr Herbert A Wenner of the Department of Pediatrics and the Hixon Memorial Laboratory University of Kansas School of Medicine will now present a paper on Sporadic Bovine Encephalomyelitis

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CHAIRMAN SHOPE Dr Douglas H Sprunt Director of the Institute of Pathology Division of Pathology and Bacteriology The University of Tennessee Memphis will now present a paper on "The Pathology of Psittacosis"

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opportunity to make these remarks on Dr van Rooyen s very excellent paper

Dr van Rooyen has reviewed the development of our knowledge of this disease very thoroughly and perhaps the best thing for me to do is to make some general comments The history of psittacosis has followed the same pattern as a number of other infectious diseases that are not very conspicuous clinically and do not have a very high mortality rate First during an unusual prevalence came the recognition of a disease entity on clinical and epidemiologic grounds Then as techniques for studying disease producing agents became available they were applied to this particular problem Unfortunately the bacteriologic techniques of the latter part of the last century only led us astray The mistake over Nocard s bacillus is an example of how a bad theory can be worse than no theory When suitable techniques had been developed for studying viruses on the occasion of the next widespread prevalence of the disease several groups of workers reported the psittacosis virus about the same time The next step was the development of a specific diagnostic test (usually the most important result of discovering the causal agent) With this tool psittacosis was found to be much more prevalent than was originally thought—as has happened with several other diseases Finally came the antibiotic era and *psittacosis became curable*

It is difficult to draw the line between history and current events but one happening that I think is worth mentioning here is the discovery that fulmar disease in the Faroe Islands is due to psittacosis One wonders whether the disease has been there all the time or whether the fulmars were infected from dead parrots thrown off ships as was suggested at the time In 1933 there were 71 cases of pneumonia which were presumably due to psittacosis eight of these people died Subsequently each year there have been between 30 to 35 cases

The story of the discovery of psittacosis in ducks and other birds on Long Island has been excellently reported in great detail quite recently in the *New Yorker* as no doubt all of you who keep up with your scientific literature know!

DR STEELE Mr Chairman I should like to direct a question to

a reference to the encephalitic syndrome There are very few lesions referring to the central nervous system Most of them as I recall were found in the peritoneal cavity and the viscera Why do you call it encephalomyelitis?

DR WENNER McNutt gave the name to the disease

DR POLLARD Are you sure you are working with the same disease?

DR WENNER Yes I think so McNutt isolated the agent in guinea pigs but he never identified it McNutt applied the name sporadic bovine encephalomyelitis to the disease Now I am not a veterinarian and I was reluctant to change the terminology at this point

As I said in my talk I do not believe that it is primarily an encephalomyelitis I think that the encephalomyelitis is part of a general systemic invasion by the bovine encephalomyelitis agent in which the viscera are also extensively involved It is our strong feeling and Dr Menges who is a veterinarian agrees with me that most veterinarians can readily identify the disease in the field simply by opening the peritoneal cavity because the fibrinous yellow web that extends over the liver and omentum (with peritoneal reflections) and over the spleen is diagnostic

DR SABIN I should like to comment on the pathology of this bovine disease based on the slides of spontaneously occurring bovine encephalomyelitis that Dr Harshfield sent me I particularly want to comment on the question that Dr Sprunt raised regarding the occurrence of demyelinating lesions in a disease caused by a virus of this sort

I was impressed that the lesions in the natural disease were so similar to those seen in measles encephalomyelitis There were lesions which were characterized by perivascular softening of tissue and cellular infiltration Although it resembled perivascular demyelinization it apparently involved more than the myelin I gained the impression that here was a virus that was attacking the blood vessels in the brain and that secondary to this attack on the blood vessels there developed the perivascular inflammatory and destructive lesions

The sections of the liver and spleen showed a dense fibrinous exudate over the surface They looked exactly like the lesions that

DR KISSLING From a diagnostic viewpoint, we do make some use of the pathology observed in these birds We can concur with Dr Sprunt that most of the cases we have seen in parrots have been acute cases and we could demonstrate the elementary bodies on a direct smear from the pericardial exudate or from the liver and spleen of these birds

In the case of the parakeets we usually do not see the acute necrosis in the spleen and liver and the pericarditis that we see in the large parrots and we also have not been very successful in demonstrating elementary bodies on direct smear preparations from these birds The parakeets that we usually receive in the lab oratory are those birds that have survived an infection and are chronic carriers These chronic carriers usually show enlarged spleens although splenomegally certainly cannot be considered a pathognomic lesion Exudation is not a common finding in carrier birds We attempt isolation by intracerebral inoculation of mice and quite often especially if the strain is rather toxic the mice may die before there is a high multiplication of virus Unless we search quite extensively—and often not even then—we cannot demon strate the elementary body but we do find an exudate in the men inges of these mice that is polymorphonuclear in character Usually the passage of a higher dilution will produce a longer period of survival of the mice and elementary bodies can be demonstrated In the mice that die quite early we find a heavy polymorphonuclear infiltration in the meninges and initial bodies can be seen but are not diagnostic due to the fact that they resemble coccus forms of bacteria

Probably this time would be best spent if Dr Wenner would like to answer some of the questions

DR WENNER We have seen elementary bodies in meningeal exudates in only two calves They are very very hard to find

DELEGATE Does the endothelial proliferation look like any other as in typhus?

DR WENNER That I can answer only indirectly because I have not worked with typhus I have a feeling that it probably is

DR POLLARD I should like to ask Dr Wenner if he doesn t feel that perhaps the name of this disease might be a misnomer because only in a minor part of the description of the disease is there

Now the question that comes up Dr Wenner is this Since you have failed to reproduce the entire picture of the disease in calves that have been injected with your agent I wonder if you can answer the question that was asked as to whether anyone has reproduced the typical disease in calves Also are you altogether justified in assuming that all of the pathology that you see in calves carrying this organism in nature (perhaps in the brain) is due to the organism?

I have a few calves on my own farm and they always have infections of one kind or another I wouldn t want to attribute anything that happens to them to an agent that I might happen to find in association with their illness

You did say that you assumed that this was a widespread infection of animals Is there some evidence for this? If that is so then it would seem hazardous I should think to assume that whatever was wrong with a calf at the time you found it infected (all the various symptoms signs and all the pathology observed) was due to a widespread agent often found in normal calves I think it may be a little bit premature perhaps in a disease as new as this to argue extensively about its pathogenic activity—particularly when disease cannot be reproduced at will by injecting the agent into its natural host

DR WENNER I probably neglected to say that after we had inoculated these experimental calves they did develop the visceral lesions

DR HUEBNER How did you inoculate them?

DR WENNER They were inoculated by different routes They were inoculated intracerebrally intraperitoneally subcutaneously and intranasally We reproduced the visceral manifestations of this disease Now we searched the central nervous systems of these animals very carefully and they do show possibly minimal signs of meningo encephalitis

DR HUEBNER Are they terribly ill?

DR WENNER These experimental calves are not very ill

DR HUEBNER Do they have fever? Are the organisms demonstrable in tissues?

DR WENNER Yes by inoculation It is very hard to find them by microscopic examination of the tissues The clinical course the

Dr Wenner obtained after intraperitoneal injection of the virus

It is highly probable that in nature the usual manifestation is a systemic infection with only occasional localization in the brain Dr Wenner s recovery of virus from the brain gives us reason to believe that one may be dealing here with a primary encephalomyelitis caused by the virus with demyelinization as part of the picture

When the techniques for demonstration of measles virus are improved we may perhaps find that measles virus also may be found in the brain There is one report already of the recovery of measles virus from the brain in post measles encephalomyelitis

Certainly it has not been possible to get vaccine virus out of postvaccinal encephalomyelitis in human beings but that may be due to the late occurrence of the disease and the presence of antibodies which are formed very early

DR SPRUNT The statement has been made repeatedly during the last 25 years that viruses will cause a primary demyelinating lesion in the brain a lesion similar to that which occurs during the Pasteur vaccination against rabies or following such viral diseases as measles It is of interest that during the psittacosis epidemic of 1930 it was reported that psittacosis caused such a demyelinating lesion Further study of the case in question however showed the lesion to be limited to the Virchow Robbins space and it did not involve the myelin sheath

Furthermore virus has not been recovered from cases of primary demyelinating lesions It is also of interest that these demyelinating lesions cannot be produced by the injection of virus into the brain Of course it is possible to get a secondary loss of myelin when the nerve cell is killed as it is in many viral diseases but this should not be confused with the perivascular demyelinating lesions

DR SABIN How about the viruses that attack the blood vessels and do not attack the nerve cells? We must keep in mind that viruses like vaccinia measles and others which can attack the blood vessels may give rise to secondary perivascular lesions in the brain which may simulate the allergic type of encephalomyelitis

DR HUEBNER In recent years we—at least I—have become very much concerned about the matter of attributing disease and all pathologic effects observed to the action of prevalent infectious agents which happen to be found in association with them

DR WENNER I have not considered this infection to be related to the typhus group

DR SPRUNT The reason I asked about that possibility is that I have heard it said that psittacosis causes a proliferative vascular lesion similar to that produced by typhus It is of course very easy to miss this lesion My main interest here is whether this lesion is perhaps related to typhus

DELEGATE I should like to ask Dr Wenner about the toxicity of the bovine encephalomyelitis virus I noticed in your discussion you said you could not reach any conclusions but how toxic is this virus in relation to some of the other agents in this group and do you have any evidence that it might transmit to man?

DR WENNER I can tell you only what Dr Meyer told me about toxin production He has one of the strains and he said it is one of the lowest toxic diseases that he has seen

DELEGATE In relation to man?

DR WENNER I do not know We bled a number of people on the farms There was no clinical evidence of illness among the handlers of the cattle and we found only a few serums that reacted in low titer The significance is not clear for they also reacted with Lygranum

DELEGATE Dr Wenner how many experimental calves have you done?

DR WENNER About six

DELEGATE I think Dr Huebner asked the question a few minutes ago as to whether anyone else has produced the disease I think that McNutt in one of his papers says that he reproduced it with egg passage virus he did not describe central nervous system lesions but I take it he was able to reproduce the disease

DELEGATE Of course this discussion should not end without raising the question of whether we really know that this virus and nature s virus are one and the same It would be a little odd to have two viruses of the lymphogranuloma venereum group affecting calves

I think Dr Wenner has left the question in his publication as to whether or not this may involve an interesting natural history of

findings at necropsy in addition to the serologic evidence indicate that at least this agent plays a role in the exudative illness of these calves

We have been intrigued with the possibility that you raised that there may be two agents in the disease but so far we have no evidence that will allow us to say that there are two agents involved

Now the reason that we said that it is a widespread disease was that we have done serologic surveys on a number of affected herds Dr Menges collected representative samples from each herd at the *time of the outbreaks and again a month to six weeks later I can* not give you the exact figures but roughly about half of the animals developed complement fixing antibodies in that period of time particularly the younger ones The adult animals may but the proportion rises and drops off

DR HUEBNER Have you tested other herds in which these illnesses are not known to occur?

DR WENNER We have tested a limited number of Kansas herds and there is very little evidence of infection in them

DR STEELE I should like to call your attention to recent information published on hog cholera encephalitis If that description had been published 40 or 50 years ago we should probably be talking about swine encephalitis today If investigators had attempted to reproduce that encephalitis with the hog cholera virus isolated they would have had a very difficult time They are still having a difficult time reproducing hog cholera encephalitis

I should like to inquire about the intranasal instillation did you suspend the organism in an aerosol or did you just drop it into the nostrils?

DR WENNER We just put it into the nostrils with a long tube

DR STEELE That may account for the difference in the observations

DR WENNER It may It probably does But we did not want to aerosol ourselves

DR VAN ROOYEN I sympathize with Dr Wenner in his difficulties It is unfortunate that the lesions were associated with encephalitis Likewise the disease does not seem to bear any obvious relationship to the typhus group

DR WENNER I have not considered this infection to be related to the typhus group

DR SPRUNT The reason I asked about that possibility is that I have heard it said that psittacosis causes a proliferative vascular lesion similar to that produced by typhus It is of course very easy to miss this lesion My main interest here is whether this lesion is perhaps related to typhus

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the virus which under certain conditions shows itself in different kinds of pathologic manifestations

DR WENNER I cannot answer that any more than a pathologist could because we have just now obtained a strain of Baker s virus

DELEGATE I have one comment on Dr Gordon s paper You mentioned salmon poisoning and the possibility that the organism was in the fish I think the work indicates that if it had the organism in the cycle it is probably in the worm all the time and the fish is just the innocent transmitter of the trematode

DELEGATE Well that would be an exception

DR WENNER Well it is a very exceptional thing to find it being carried by a trematode too

Afternoon Session, First Day

Dr James H Steele United States Public Health Service Atlanta Georgia presiding

CHAIRMAN STEELE Dr Geoffrey W Rake Medical Director The Squibb Institute for Medical Research New York City will now present a paper on 'The Diagnosis of Psittacosis

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DR DORLAND DAVIS I should like to ask Dr Rake what he thinks about the diagnosis of psittacosis in patients who have received early and rather adequate antibiotic treatment How does that complicate the diagnosis either by serology or by isolation of the virus?

DR RAKE I think Dr Davis you may be able to answer that question better than I can Again I would point out that in the days when we were most actively working with this group we did not have the drugs available that we have today

Now in one case of a break in laboratory technique a pipette became unplugged or a pipette was not plugged and the girl who was doing the transfer sucked on it thinking that it was plugged and sucked a whole lot of material from the pipette into her mouth We knew that the break in technique had occurred We started treating at once with sulfadiazine She never had any real symp

toms She had a little fever on two days and a complement fixation test did become positive I believe her highest titer was about 1 32 and then it became negative again

Now in those days at least as far as our experience was concerned the complement fixing titer in patients who became infected with a member of this group always stayed high either because we never did sterilize their tissues (which has always seemed to me to be the case) or for some other reason This was particularly true when treatment was not started until late

In my own case I had an infection which was not diagnosed by the clinical group—although I told them what I had—until a week had gone by I was then started on sulfathiazole in extremely small and cautious doses which did nothing at all

I have a titer today of about 1 2 000 It stays at that level and no matter what I do nothing changes it

I believe that when drug treatment is successful particularly in the earlier stages it is possible to a large degree to prevent complement fixing antibodies from appearing Certainly today with some of the drugs which are available some effect can apparently be obtained—because in the small proportion of the cases which we have seen it was possible to bring the complement fixing titer back to zero

DR POLLARD We have recently had a small epidemic of psittacosis in our laboratory Realizing what was happening we started treatment and studied the complement fixation reactions on all of our cases

The first case was not treated and he had a prolonged illness which produced a peak titer of about 1 640 It is still up around 1 40 after a year The second one was treated a week after onset and his peak titer was about 1 600 and the third one was treated on the first day of illness and the highest peak he reached was 1 40

DR RAKE Did he clear up?

DR POLLARD We haven t followed it that long It s a current epidemic

I should like very much to accept your decision regarding the specific skin sensitivity of individuals to the particular virus with which they are infected But we had an occasion to check some cases of lymphogranuloma venereum (LGV) with psittacosis

virus and I must say that the skin test reaction we observed was severe enough to dissuade most of them from taking a re test It was a very severe reaction with lymphadenitis

DR RAKE Did you use a control antigen?

DR POLLARD Yes

DR RAKE I have not encountered such a series

DR POLLARD These were acute LGV cases

CHAIRMAN STEELE I should like to inquire of Dr Rake if the skin sensitivity tests can be used by a physician if the patient has received antibiotics immediately

DR RAKE In my experience if the drug treatment is started early the skin never becomes positive The complement fixation test may but the skin test never

CHAIRMAN STEELE Have you had any experience with the skin test in animals?

DR RAKE Yes In mice we never got anything We observed a slight reaction in guinea pigs but it was not highly satisfactory

CHAIRMAN STEELE Dr Kissling you have done some interesting work in the past year on the isolation of the virus from the droppings of bird cages and I wonder if you want to describe how you handle your material and what your over all success has been

DR KISSLING We treat our material with streptomycin alone (sputums are also treated that way) and we always inoculate intracerebrally when we can We know that mice are much more sensitive indicators of the virus when inoculated intracerebrally rather than intraperitoneally

DELEGATE Do you have any special reason other than to differentiate between lymphogranuloma and psittacosis for giving the virus intraperitoneally?

DR KISSLING We obtain our diagnosis at least three or four days earlier by the intracerebral route than we would by the intraperitoneal

DR RAKE I think that is a good point We always did it intracerebrally because our work started with lymphogranuloma We were always very sensitive to the lymphogranuloma problem I think you will find though that in the techniques by Meyer in

Francis book on laboratory techniques for virus diagnosis the intraperitoneal technique is recommended

DR DAVIS It has been our practice in trying to isolate the virus to inoculate both intracerebrally and intraperitoneally and the prime reason for inoculating intraperitoneally is to get a larger inoculum One can inoculate 0 5 cc or 1 cc intraperitoneally whereas 03 or 05 cc is the maximum amount that can be injected intracerebrally Actually I do not know whether or not the results are any better If the virus is there we usually get it out very quickly intracerebrally

DR POLLARD May I ask Dr Davis if he has ever isolated a pigeon strain from an intraperitoneal inoculation alone

DR DAVIS We have isolated pigeon strains by inoculating mice first intraperitoneally then passing the mouse spleen liver and kidney intracerebrally

DR POLLARD The mice never do die?

DR DAVIS They never or rarely die when inoculated intraperitoneally

CHAIRMAN STEELE Dr Kissling what has been the percentage of your isolations from the cage droppings?

DR KISSLING Actually not too high I think they have not been well selected They have been more or less of a survey nature

The most interesting one we ve had probably was a little piece of filter paper with a little soiled place in the middle of it sent in for a complement fixation test It had been in several different laboratories for a long time and we wondered if we would be able to get any virus from it but sure enough there was still some virus there It had never been refrigerated But the fact that it was well dried probably preserved it

CHAIRMAN STEELE Dr J Vernal Irons Director of Laboratories Texas State Department of Health Austin will now present a paper on Psittacosis in Turkeys and Fowls as a Source of Human Infection

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CHAIRMAN STEELE I think we all owe Dr Irons our commendations on proving that this was a serious problem in turkeys

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DR KISSLING We obtain our diagnosis at least three or four days earlier by the intracerebral route than we would by the intraperitoneal

DR RAKE *I think that is a good point* We always did it intra cerebrally because our work started with lymphogranuloma We were always very sensitive to the lymphogranuloma problem I think you will find though that in the techniques by Meyer in

a picture of the nature of the hazards of the various processing activities as regards the rate of infection The employees engaged in removing the pin feathers from the birds had the highest rate of infection as 18 of the 32 workers in this department were involved There were no infections among seven workers engaged in grading and packing activities and only one out of 20 among trucking receiving and miscellaneous workers As would be suspected the workers subjected to the greatest contact with the birds showed the highest rate of infection The fact that 22 out of 78 workers became infected in this outbreak or nearly 30 per cent emphasized the epidemic proportions of psittacosis in this establishment

Some of us were not sufficiently convinced that the outbreak of 1948 was actually psittacosis since the diagnosis was based on the serologic studies of the patients and no isolations of the agent had been made either from the turkeys or the human cases From what we know now and as Dr Irons and his co workers were convinced at the time the serologic picture presented was significant

The second epidemic Dr Irons described occurred in December 1951 Of 134 employees there were 44 cases of psittacosis with four deaths As in the 1948 epidemic careful investigations again indicated turkeys as the probable reservoir of the infections Of particular interest was the observation of re infection among the workers who had psittacosis in the 1948 epidemic Twenty two of the workers infected in 1948 were studied in the 1951 outbreak Eight of the 22 workers were involved in picking and eviscerating activities where the incidence of exposure was greatest Three out of this group of eight became re infected which raises doubt as to the duration and extent of immunity Nine of the workers were apparently exposed but escaped infection in 1948 however they became involved in the 1951–52 epidemic Two of the four deaths in 1951–52 were among 1948 workers who supposedly escaped infection at that time

The distribution of cases was greatest among picking room workers 56 6 per cent the eviscerating workers 25 0 per cent *two out of three workers* 66 6 per cent doing picking and eviscerating work and three out of 20 15 per cent among the pre processing workers Again the incidence of disease was highest

The successful follow up work by Dr John P Delaplane Head of the Department of Veterinary Bacteriology and Hygiene Agricultural and Mechanical College of Texas is also a very important contribution Between them they have developed much new valuable interesting information I know it will stimulate many questions about turkey psittacosis

Dr Delaplane will now present a discussion of Dr Irons paper

DR DELAPLANE It can be seen that the greatest number of probable sources of human infections in the Texas outbreaks implicated turkeys although from 1935 to 1953 parakeets parrots pigeons man and chickens had been found as possible sources of infection in Texas

Since the greater effort of Dr Irons and his colleagues has involved psittacosis occurring in poultry dressing plant workers it may be well to call attention to the points which I as a veterinarian interested in poultry pathology would consider of particular interest

The Malone Texas epidemic of 1938 involving seven cases with four fatalities leaves room for speculation regarding the probable source of infection such as the contact with the sick calf or possibly with turkeys or chickens since there was no known contact with psittacine birds thought so essential at the time as the source of infection It is of particular interest that until 1948 only four laboratory confirmed cases of psittacosis had been diagnosed in Texas Since then some 114 human cases have been recorded I think Dr Irons would agree that this perhaps does not represent a marked increase in the occurrence of the disease but that it reflects the difference between being on the alert to the study of suspicious cases and an attitude of indifference to it

In October and November 1948 the first of three outbreaks of psittacosis occurred in the workers of a poultry dressing plant in Texas There were 22 cases and three deaths among 78 employees of the establishment Serologic studies showed complement fixation titers using lymphogranuloma venereum antigen reached and maintained high levels for weeks Epidemiologic studies indicated that turkeys were the probable source of infection since turkeys were processed during the time necessary to account for the human exposures Further epidemiologic investigations give

a picture of the nature of the hazards of the various processing activities as regards the rate of infection The employees engaged in removing the pin feathers from the birds had the highest rate of infection as 18 of the 32 workers in this department were involved There were no infections among seven workers engaged in grading and packing activities and only one out of 20 among trucking receiving and miscellaneous workers As would be suspected the workers subjected to the greatest contact with the birds showed the highest rate of infection The fact that 22 out of 78 workers became infected in this outbreak or nearly 30 per cent emphasized the epidemic proportions of psittacosis in this establishment

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where exposure to the birds was greatest from direct contact and droplet infection An infection rate of 33 6 per cent of the employees and four deaths emphasizes the importance of the infection to poultry dressing plant workers The data indicate that *probably the higher attack rate in females was attributable more* to exposure due to the nature of the work than to greater susceptibility in comparison to males The attack rate among Negroes was also probably due to greater exposure rather than to greater susceptibility as compared to whites

Investigation of the third epidemic in this same plant in April 1953 again pointed to turkeys as a probable source of the infection The epidemiologic picture was similar to the previous epidemics except that more white women than Negroes were involved because relatively few Negro women were working Most of the white women involved in this outbreak had worked during one or both previous epidemics but Negroes had been primarily involved in the picking activities on these previous occasions

The laboratory findings in the three outbreaks failed to show anything consistently significant except for the complement fixation studies with LGV antigen which showed the serums from the patients with sharp rises in titer to the LGV group of agents Similar results were obtained with psittacosis antigens While rising titers were not obtained during the first epidemic of 1948 the majority were high and persisted for weeks In the second and third outbreaks typical rising titers were obtained from a number of the cases Titers were observed in individuals who were not ill indicating exposure without clinical manifestations Unfortunately specimen material from the cases such as sputum and throat washings failed to result in the isolation of the agent perhaps the use of antibiotics had interfered in this respect since such therapy was usually under way before the specimens were obtained

Perhaps the most important finding to support the suspicion of turkeys as probable sources of infection came as a result of the isolation of the agent from a turkey on Farm A which had been under investigation as being involved in the 1951–52 epidemic An undiagnosed illness in turkeys on Farm A before and after the 1951–52 epidemic helped to focus attention on the flock For example sick birds presented to the poultry diagnostic laboratory

had included some birds with pericarditis cloudiness of the air sacs or enlarged spleens which failed to reveal anything of a bacterial nature although infectious sinusitis as an agent had not been eliminated as a possibility

Two birds from this farm obtained April 10 1952 were presented for autopsy both birds had cloudy thickened air sacs enlarged spleens and a fibrinous pericarditis—lesions incidentally observed with infectious sinusitis or the chronic respiratory disease now known to be caused by a pleuropneumonia like agent White mice injected intracerebrally with tissue obtained from the turkeys died after a short incubation period The agent was also lethal for chick embryos and guinea pigs from which Dr Irons found abundant elementary bodies typical for the psittacosis agent

Of further interest in this connection is a human laboratory infection contracted from working with the same turkey tissue material In February 1952 before starting the studies a serum sample was negative for psittacosis but following recovery from an infection in August 1952 the patient gave a titer of 1 96 as reported by Dr Irons Other human laboratory infections occurred among workers involved with studies of this same turkey source material

The isolation from turkeys of an agent capable of infecting man lends further confirmation to Irons suspicion that turkeys were the probable source of the infection in the Texas poultry dressing plant

Of interest is the case of psittacosis probably acquired from eviscerating chickens in Nebraska as indicated by the serologic studies involving the migrant Negro worker on her way to pick cotton in Texas This would indicate that psittacosis will probably be found nationwide if diligent efforts are made to isolate it from both turkeys and chickens I for one refuse to concede that Texas has a monopoly on the disease as it occurs in poultry Much information is needed to describe the pathology of psittacosis as it occurs in the chicken and turkey so as to serve as a guide to the veterinarians responsible for poultry meat inspection activities in the United States Serologic studies of employees in poultry dressing plants are needed to serve as an index to the possible extent of such infection throughout the country

From what little is known regarding the lesions seen in turkeys these lesions could well be mistaken or masked by the air sac dis ease problem Perhaps research could do much to define differ ences in this respect

As seen in Tables XI and XII CFI titers were observed in fowl from the various farms without being indicative of any particular one that could have served as a source of infection

DR STEELE In attempts to reproduce the disease did you inoculate the turkeys via the frontal sinus as is usually done in reproducing chronic respiratory disease?

DR DELAPLANE We attempted to inoculate turkeys because at that time we were not sufficiently satisfied that infectious sinus itis might not be tied up somewhere in the picture We ordinarily bring about sinusitis infection by inoculating the sinuses We in oculated some birds by the intrasinus route and there were no visible clinical effects however when they were killed and ex amined we observed enlarged spleens When we inoculated young turkeys via the air sac route they showed symptoms in about eight days characterized by fever prostration and death From what we have seen we might well be suspicious that the lesions could be confused with those observed in the lower form of in fectious sinusitis

DR IRONS Dr Delaplane has referred to Tables XI and XII which I did not show because of the lack of time They concern complement fixation inhibition or indirect complement fixation tests on bird bloods You might show the last two there if you will [see pp 59–60 in text]

DR DELAPLANE These tests did not help us very much

DR IRONS No because on an epidemiologic basis we had one particular flock pinned down but actually here in addition to Farm A we had Farms B C and D which were the farms from which the biggest flow of birds came Now some of these brought in only chickens—no turkeys—but when we tested as many as seven or eight ducks geese chickens and so on from each one on three of the four farms we found complement fixa tion inhibition titers

Dr Meyer did even better than that You people sent him specimens from five farms and you got turkey bloods from way

over in the other part of the state (well over in the bovine en cephalomyelitis country Dr Wenner) rather than in the turkey ornithosis country as we understand it

Now lets look at the titers We ran them three or four times in different serologic set ups—one we called a relatively insensi tive test one a sensitive" and one a very sensitive test What I have given you here is a conservative approach because they are not "very sensitive In the very sensitive tests we had some complement fixation inhibition titers that ran very high

But on the turkeys only two of the 14 really showed very much in the way of complement fixation inhibition titers Notice the chickens We also found a fairly good titer in a chicken from an other flock And we found a good titer in one duck and a very good one in a goose And two titers here were from guineas

I want to say a word about this We were concerned with this particular flock because of chickens—a lot of 70 chickens that came in on the day which we believe was the exposure day for the second epidemic When we went out to the farm we found a lot of different kinds of fowl The owners were two sisters old maids who had an old mother who just had to have various kinds of bird meat to eat She didn t like beef or pork but she liked all kinds of bird meat so they had most any kind of domestic fowl you could mention They didn t have pheasant That was the one thing that they didn t have

So we really had an opportunity there in serologic studies and most of our positive findings came from that flock I don t know what you would find if you were to go out studying other similar mixed flocks I suspect though that you would probably find the same sort of thing elsewhere and maybe in other states too

It is all very interesting but I don t know what it means We had considerable difficulty getting the same results in our laboratory that Miss Eddie got in her laboratory In fact I discovered that my serologist and Miss Eddie use almost different principles of serology They are both good serologists but neither one could understand the other s controls!

It would be rather complicated for me to try to explain to you how all this works out but I must say it is not very satisfactory to try to use the complement fixation inhibition or indirect approach

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DR GOSS You used six birds altogether?

DR DELAPLANE Yes We should have used more but the danger of human infection incident to work with the live birds caused us to elect the use of embryonating eggs But it so happens that my infection dates from working with eggs rather than from working with these birds

DR GOSS Did I understand you correctly that this agent killed guinea pigs?

DR IRONS Yes it did kill guinea pigs

DR GOSS By what route?

DR IRONS By the intracerebral route and I believe we had some younger guinea pigs that we killed by intraperitoneal inoculation too

DR GOSS Consistently?

DR IRONS Yes I believe so I believe a good dose would kill them rather consistently

DR DELAPLANE Dr Meyer reported that this was one of the more toxigenic strains Is that not correct Dr Irons?

DR IRONS Yes he told me that he believes this is one of the most toxic viruses that he has ever worked with We had quite a discussion over at the American Public Health Association meeting as to what is meant by a toxic virus" and what is meant by a "toxin in association with a virus I should suppose that the general properties of this virus would fit in fairly well as I understand it with the virus which Dr Kissling recovered in Louisiana from the egret When we speak of southeast Texas we are referring to an area near Louisiana and it seems likely that our turkey virus is closely related to that recovered from the egret

As you know poultrymen and turkeymen go to great trouble to keep turkeys away from the chickens to prevent blackhead but turkeys on the range are not protected against exposure from all kinds of wild birds Pigeons I think were pretty much discounted We thought of them in particular but on the farms that were so highly suspect there were no pigeons around There are doves all over Texas but we haven t examined them for the infection which has been found by others in this species There are many kinds of migrating and other birds that could have been the source of infection for turkeys

We have had the experience of testing a certain flock of pigeons one day and perhaps finding a pigeon that had a good titer A few weeks later we couldn t find a single pigeon that had a good titer Sometimes pigeon serum was stored in the ice box and lo and behold a couple of weeks later the titer was badly deteriorated

I think Dr Davis told me that he had some similar experiences This is about the only serologic approach that we have investigated Perhaps Dr Hilleman who has had a lot of experience in some of the other laboratory tests could suggest the proper approach to work out a blood test and some practical approach as well so that the poultry pathologists might benefit But I still don t know how we can correlate and interpret all the findings and the epidemiologic findings just do not fit in well with the over all serologic picture

DR STEELE Well to make a long story short you and Dr Meyer found a variable percentage of reactors in almost any group of turkey samples—sometimes as high as 30 per cent

DR GOSS What is the mortality in these turkeys from either natural or artificial infection?

DR DELAPLANE That was one of the pictures that Dr Irons pointed out here a moment ago at the moment it is very difficult to interpret We know that the owner brought birds to the laboratory on four or five occasions before we became suspicious and *we might assume that he must have had appreciable losses* otherwise he would not have been interested in coming back to the laboratory

One of the reasons we failed to check for other than bacterial agents before that was that we ordinarily use the turkey as our assay or laboratory bird for turkey sinusitis and we were storing the material for later studies of that kind We had not completed these studies at the time this came up

DR GOSS Were you able to reproduce the disease?

DR DELAPLANE In the turkey? Yes

DR GOSS What was the mortality in those birds?

DR DELAPLANE Of the first four that we inoculated by the intrasinus route two older birds showed very definite lesions Of the last two one died on the eighth day the other one was morbid and was killed

pound is the minimum and I am sure that most turkeys do not get anywhere near that

CHAIRMAN STEELE Dr Levine and I can get together later and review the Public Health Morbidity Reports for the first three months of this year which report some 30 home food outbreaks where turkeys are mentioned

CHAIRMAN STEELE Are there any further questions to be directed to the speakers on psittacosis?

DR. POLLARD Just briefly may I say that Dr Delaplane sent the turkey strain to us and we inoculated it intramuscularly into several dozen chicks It killed every one of them in five days We inoculated week old ducklings intramuscularly and it killed every one of them in six days In attempting to study a viremic pattern we inoculated older birds which were held as long as 28 days but they died We never did have a survivor from that one strain Previously we had tried the pigeon strain P-4 and observed a non fatal viremia in the same species of birds

DR GORDON Dr Irons remarks about the possibility that psittacosis in turkeys may have a depressing influence on egg laying I think is very interesting in view of the possibility that eggs may be infected Infected eggs of domestic fowls could possibly serve as a source of infection for man and also perhaps effect congenital transmission in fowls Does Dr Irons or anyone else have anything more to say on the presence of the virus in eggs from infected fowls?

DR DELAPLANE In connection with egg transmission I should say that considerable research work is needed because even with agents that we can readily isolate it is difficult to get proof of egg transmission We have considered the idea

DR LEVINE Just one practical observation with reference to the public health importance of turkeys and psittacosis From an inspection standpoint by the time the bird has reached the poultry meat inspector the damage has already been done insofar as human exposure is concerned

DR DELAPLANE Yes but in my opinion serologic surveys of poultry dressing plant workers would serve as a guide to the presence or absence of infection in the country or poultry population

DR SHAUGHNESSY These comments on the complement fixation inhibition tests are of interest because we are currently having an outbreak in a small community in the northwestern part of Illinois It is certainly not due to psittacine birds and the question arose as to whether it was coming from chickens

Blood samples from chickens were sent to Dr Meyer for complement fixation inhibition tests and his laboratory reports a fairly high proportion of positives from a number of flocks

While I m speaking I should also like to point out that turkeys are very good disseminators of *Salmonella* organisms even more so than chickens Since turkeys also seem to be good reservoirs of psittacosis virus and since many of the older investigators mentioned the fact that parrots and other psittacine birds that were infected with psittacosis were also apt to be carriers of *Salmonella* one would wonder whether there is any relationship between the two diseases

DR LEVINE I m afraid that I as a poultry pathologist cannot take Dr Shaughnessy s remarks without raising at least a single voice of protest The *Salmonella* problem could I think bear closer scrutiny and I still feel as I always have that despite the fact that turkeys do harbor *Salmonella* organisms in their intestinal tracts that in itself does not make them disseminators of salmonellosis to the human population

I should like to see concrete data to show that fowl suffering from a *Salmonella* septicemia was the cause of human infection after this fowl was prepared for food and prepared as poultry meat normally is in this country well done

DR SHAUGHNESSY I should like to say that we have investigated several outbreaks of salmonellosis one involved almost a thousand cases in which turkeys were incriminated In this outbreak the turkey was served at a church dinner and the leftovers were served the next day to a group of school children and they as well as those who had attended the dinner came down with salmonellosis In most homes and other places turkeys are often stuffed the day before and kept in a room which is not cool enough for refrigeration so that the dressing serves as a beautiful culture medium Then due to improper cooking *Salmonella* may escape destruction I think all the cookbooks tell you that 20 minutes per

ease (C R D) agent I believe you have reported that it is primarily a granulomatous type of pathology and I believe this morning we heard that that was more or less true with psittacosis What would you think of the problems involved Dr Young?

DR YOUNG From the gross pathologic viewpoint it is practically impossible to make a definite diagnosis The microscopic picture of the serous membranes is similar in the C R D group of diseases and psittacosis The virus or inclusion bodies should be looked for in the lesions observed You have to consider such diseases as Newcastle disease and infectious bronchitis of chickens occurring separately or concurrently with the C R D group or ornithosis I believe we will eventually have to resort to differentiation by serologic tests

Dr Delaplane has mentioned that C R D has especially in the most typical cases a very definite granulomatous pathology more noticeable in the air sacs and the pericardium

Pericarditis in turkeys and in chickens is certainly not always caused by the C R D agent Newcastle disease and the ornithosis (psittacosis) agent usually cause a pericarditis thus there are several other agents which may cause these changes When lesions of this type are present one should always be suspicious of the presence of these various agents

CHAIRMAN STEELE Dr Dorland J Davis Chief of the Laboratory of Infectious Diseases National Microbiological Institute National Institutes of Health United States Public Health Service Bethesda Maryland will now present a paper on "Psittacosis in Pigeons"

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CHAIRMAN STEELE Dr Robert F Korns Director Bureau of Epidemiology and Communicable Disease Control New York

DR LEVINE Would it not be better to make surveys of turkey flocks?

DR DELAPLANE We should first have to convince some of the poultry pathologists who are as skeptical as I was about Dr Irons work Some of my colleagues were more hostile than I was in that respect But I think that a survey of the type mentioned would convince poultry pathologists

CHAIRMAN STEELE I believe that Dr Irons found that the veterinary inspector at the Giddings plant suffered a virus pneumonia illness for three weeks When he returned and heard about this psittacosis outbreak he asked Dr Irons to test his blood What was his titer at that time?

DR IRONS He had a good titer 1 64 or 1 128

CHAIRMAN STEELE Was he not quite surprised to learn that he had had psittacosis?

DR IRONS Yes indeed You see this illness occurred just before Christmas and afterwards he went to Houston and of course had no more contact with the people at the plant On returning he was amazed to learn not only that he probably had psittacosis but that there were 43 other employees who had it too Of course he knew he had had a very serious illness

DR IRONS I might add and it s rather pathetic in a way that I frequently get bits of turkey tissue from a Quartermaster Depot or a pathologist with no mention of psittacosis but I know what examination is wanted I know there are a number of pathologists and inspectors who become suspicious and probably have an uneasy feeling when they inspect birds which have sinusitis or air sac disease

Now if we could give them more clues perhaps we could help them I personally wish the inspectors would pay a little more attention to the heart particularly for evidence of pericarditis and also for air sac exudate However I am not enough of a poultry pathologist to know if these changes would be of practical value as guides Of course the damage is already done since the inspector is about the last man in the line to be exposed

DR DELAPLANE In regard to the pathology I think it might be a good idea if you Dr Young would say a few words about the problem involved in connection with the chronic respiratory dis

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State Department of Health Albany will now present a paper on "Psittacosis in Ducks and Persons Exposed to Ducks"

• • •

DR SABIN There is something peculiar about the high incidence of virus isolations from almost every species tested Did you have any suspicions about the high incidence of virus isolations?

DR KORNS No you are the first to raise doubt

DELEGATE I should say the feelings were expressed by the duck growers

DR KORNS Yes there certainly were such feelings among the duck growers and that presented a major political issue We had great difficulty in collecting specimens because they would meet us with armed force to prevent it

DR SABIN I think that the New York State Health Department has a very fine Division of Laboratories Now if you think that the incidence of ornithosis in Long Island is so high that you can just pick a couple of birds and get 30 to 50 per cent virus isolation it should not take more than a week s work in your Division of Laboratories to confirm that

DR POLLARD Well what can you gain by isolating the virus? Everyone knows that the ducks are infected

DR SABIN That is just what I don t know

DR POLLARD You will find that virus in every species if you look long enough

CHAIRMAN STEELE The point that Dr Sabin is making is can you at random selection expect to isolate virus so frequently

DR SABIN I am not saying that ducks cannot have ornithosis they can have it But I doubt whether it is possible to pick four ducks and get those results at random if that is true I think it ought to be confirmed

DR SHAUGHNESSY Partially in self defense—because when I appear I shall present some data of this kind—I may say that we have tried to isolate this virus from groups of chickens or from groups of other fowl at times with no or a very low incidence of successful isolations as contrasted with what we found in pigeons Also we have seen no evidence of infection in our laboratory am

mals although we have looked for it And when we try to isolate virus from people we often get a very low percentage of isolations probably because of the types of specimens we receive I hasten to say in these days

DR HUEBNER I have been in a number of different virus investigations in the last seven or eight years and in nearly every one at the time that we got into them it was generally assumed that if one isolated a virus from a field specimen one would accept it at its face value—assume in other words that it represented a highly significant observation

In the rickettsial field we didn t question that either However in going over the literature it seemed unusual to find that any laboratory looking for Q fever in ticks would always find it quite promptly It made no difference what country it was or what species it was or in what laboratory the agent was always found

I often asked these laboratory people if they had ever seen any spontaneous infection in their laboratory animals? The answer was always negative but nobody did any studies to find out I didn t do it either until we had an outbreak in our laboratory in 1946 We had a sizable number of human infections half the staff came down with Q fever In attempting to isolate the organism from some of these people we often failed to demonstrate it by injecting it in our guinea pigs under circumstances which seemed ideal for that purpose

Then for some reason or other we thought we would bleed all the guinea pigs in the laboratory and depending on where they were in the building we found that 10 to 30 per cent of the guinea pigs had Q fever antibodies they had already contracted Q fever

We asked other laboratories about this potential problem but they still did not have any problem until we insisted that they check their guinea pigs and when they did they found Q fever occurring spontaneously in their guinea pigs too

In our Q fever studies in Los Angeles we examined about 2 000 control guinea pigs which were never injected with anything but were carried along in the same cages with injected animals We found that the intensity of our work and the dose of *Coxiella*

burneti used as inoculum determined the amount of spontaneous Q fever in our laboratory When lethal dosages were injected into test guinea pigs we produced Q fever almost invariably in the control animals kept in the same room So, the results of three years work and observations on several thousand uninjected guinea pigs suggested that if we would inject 100 guinea pigs with field specimens from anywhere we couldn t fail to isolate one or two strains of Q fever provided it was done in our base laboratory where a great deal of work was done on Q fever Now psittacosis we have to remember is a very similar agent It moves around in the air and therefore will get to experimental animals If it does so in nature why shouldn t it in the laboratory? I feel sure it must do so However, whether or not they become infected is another question

I should like to see more evidence based on virus isolation attempts of the possibility of naturally susceptible hosts developing spontaneous infections in laboratories Certainly in the psittacosis field this would be indicated Definitive studies to determine whether spontaneous infections occur in the laboratory seem almost never to be done This is unfortunate when so much depends on the validity and significance of virus isolation performed in contaminated laboratories

We had a $2 000 000 laboratory that we had just built a beautiful laboratory We thought we should not have any more spontaneous infections We were all right for the first three months then the infections started However we also had a garage in Los Angeles an open garage and we had nothing but lard cans available for animal cages We found that control guinea pigs in this improvised laboratory never did contract Q fever—and we never failed to bleed a guinea pig before discarding it Characteristically we had no trouble at all in an environment where the organism was not being worked with in concentrated amounts

DR COTTRAL In defense of Dr Meyer I may say that at the United States Regional Poultry Laboratory in 1948 we sent Dr Meyer about 25 serum samples from our birds and he did not find evidence of ornithosis in any of them!

DR SUSSMAN Considering the numbers of ducks that are sent throughout the country from the supposedly highly infected

area why haven t many other people gotten psittacosis simply by handling the ducks in the kitchen prior to cooking

DR KORNS Dressed duck? Or the whole live duck?

DR SUSSMAN Well either one

DR POLLARD May I ask Dr Korns first if the specimens which were sent to Dr Meyer were collected with separate instruments

DR KORNS Yes Dr Meyer and Dr Eddie of his staff came to Long Island and collected some of these specimens but the great majority were collected jointly by Dr Eddie and our own team of which I was a member I can say that a rather meticulous technique was used—separate instruments and the specimens were packaged and frozen in such a way that they would not be cross contaminated

DR HUEBNER I do not think we could question those results—it is only that one would expect some variation in percentage 10 per cent in one group and 95 per cent in another There is the possibility that in psittacosis as in some other diseases there may be a situation of maximum exposure but only a certain proportion of them the new susceptibles are regularly found to be infected because of the ecology of the disease in the area I think that could happen

DR IRONS I should like to tell you that we knowing that Dr Meyer had found a very high percentage of positive complement fixation inhibition tests on turkey bloods from areas which we were not epidemiologically suspecting as being involved in the turkey ornithosis in Texas wondered when we sent him a number of tissues if he wouldn t isolate a virus I can say that he never did find the virus until we told him we had found it in a turkey and sent that turkey tissue to him So as far as the turkey tissues were concerned we had no disagreement Nor did he succeed in finding the virus in human blood clots in which we were unable to find it He assured me that I would be very fortunate to get it from the human blood clots unless I could get samples from people who were not already on therapy

With regard to Dr Huebner s comments I may say that we had a similar experience We wondered about these reports on the recovery of Q fever out of ticks and we spent quite a sum of money

in Texas investigating the basic factors involved in tick transmission of Q fever in an area that we knew to be rather heavily seeded We acquired information on which cows and which herds were infected as well as about a number of people who became infected and finally about the infection of goats We tested many lots of ticks and worked almost three years before we ever found ticks infected with Q fever And when we did we began finding it repeatedly and this happened because one of our men was engaged in trying to transmit the infection by means of ticks We had very heavily infected ticks around the laboratory and we began to find a very high percentage of significant titers in our guinea pigs which never before had shown titers So I am very cautious too about accepting some of the reports of findings on Q fever in ticks when I know that in our experience it has been so very very difficult to demonstrate it in the field We have had experience with other accidental infections too

Morning Session, Second Day

Dr H J Stafseth Head Department of Bacteriology and Public Health Michigan State College East Lansing Michigan presiding

CHAIRMAN STAFSETH Dr Howard J Shaughnessy Deputy Director Division of Laboratories Illinois Department of Health Chicago and Department of Public Health University of Illinois School of Medicine will now present a paper on Psittacosis in Wild Pigeons

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DR POLLARD There is one additional report of an outbreak of psittacosis from pigeons by Macrea in the *Journal of the Royal Sanitary Institute* in 1951 in which he refers to two cases of psittacosis in people who were engaged in preparing pigeons for food I think this should be added to the record

DR SHAUGHNESSY I didn t attempt to include cases due to domestic pigeons There were a good many of these

DR POLLARD Macrea didn t specify whether they were domestic or otherwise

DR SHAUGHNESSY Well I have found a couple of out

breaks also in which it was not specified whether the pigeons were wild or domestic and I preferred to leave them out for lack of information

DR HUEBNER Is the domestic pigeon industry large enough to provide a fairly large group of industrially exposed persons? If you have evidence here and it would appear that you do of a high prevalence of psittacosis and ornithosis in pigeons a study on an industrially exposed group might be carried out At least these people might show serologic evidence of previous infection and some evidence of excess illness as the result of exposure

DR POLLARD How can psittacosis be differentiated from lymphogranuloma venereum?

DR HUEBNER That would be a very serious problem but not insuperable there should still be more positive reactions in intensively exposed persons I am thinking of similar studies that have been done in other fields where by working from the known infected animal to exposed humans it was possible with suitable tests to determine on the basis of intensity of the exposure the potential importance of the suspected reservoir

Now if one found for instance that an adequate sample of persons who were industrially exposed to infected pigeons gave no evidence of prior infection with psittacosis such a result might have a bearing on determining whether or not pigeons are important in the spread to man—which after all is the real problem implied in this discussion

From your results last year in Chicago despite the thousands of persons who had been exposed apparently people were not hospitalized with psittacosis as a result of the exposure to pigeons Maybe pigeons do not serve as a good reservoir from which to spread psittacosis There are many agents rickettsial agents particularly that can be found in all kinds of natural hosts perhaps as many as a hundred different species all over the world and yet most of the hosts are not involved in the spread of the agent directly to man

DR SHAUGHNESSY I have no personal knowledge of the extent of the domestic pigeon industry I should imagine in some areas it is fairly extensive and also though we have not investigated that phase of the problem it would appear from the few

reports in the literature that there is a fairly high incidence of psittacosis in those who are exposed occupationally to pigeons There are many references to people who raised domestic pigeons and who developed psittacosis or in some instances the disease even developed in people who were in the vicinity of the pigeon lofts

I recall one instance of a man who just liked to watch his sons raising pigeons and after he had been visiting them for a few days he caught the disease It is still hard to understand why, if this is an important reservoir there aren t more infections due to this source unless these are very mild and do not come to attention

DR GOSS I think that I might try to answer that question partially I have been amazed at the number of pigeon fanciers in the Metropolitan New York area There are literally thousands of them It seems as though on the top of every apartment house in the Bronx there is at least one pigeon loft and the owners are in there every night The first thing they do when they come home from work is to go to their pigeon lofts and handle the birds The exposure must be terrific Many of those birds are kept under the most adverse conditions I would think

These fanciers work very intimately with the birds As an example the real fancier who has squabs in the nest will supplement the feeding as the parent would if they are not doing well that is by chewing up grain in his own mouth and forcing it into the squab s mouth A closer contact than that would be difficult to effect especially when the parent birds have been doing the same thing to the squab during the course of the day

It would be very simple I believe to conduct an investigation into the incidence of psittacosis in these fanciers and believe me there are literally thousands of them Other people who come in intimate contact with pigeons are those who fly and train the birds There are people in the business who make rounds by truck every day to various lofts picking up a dozen or more pigeons from each loft they put as many as 2 000 such birds in their trucks and drive them over to Jersey where they turn them loose to fly back Such people as that I think would give a fair index as to the importance of the disease in racing pigeons at least

DR HUEBNER There might be some problems in that respect

I should like to point out that you would still have to get specimens from the fanciers You could get a history probably but still you would have to knock on the door and ask for a specimen

DR GOSS I think you could get it very readily

DR HUEBNER You may But different categories of the people vary in that respect—we have had a great deal of experience with such an operation For instance in Los Angeles we sampled 10 000 persons by going from house to house and asking for a blood specimen Our percentage of success varied from as high as 75 and 80 per cent in the middle class fairly well educated groups to about 40 per cent in the poorer groups and zero in Beverly Hills It made a lot of difference where you were working as to the amount of cooperation that you could expect

DR STEELE There is a recent publication from Denmark by Blorsom on the study of psittacosis in pigeon fanciers He makes a very definite point of saying that there is no lymphogranuloma venereum in Denmark so far as is known The serologic survey indicates that a sizable number of people are carrying a positive complement fixation titer but as Dr Huebner points out, it is very difficult to get any history of disease in these people But it is interesting that they do have a high prevalence of complement fixing antibodies

DR GOSS I think there is another aspect of Dr Shaughnessy s paper that deserves some comment and that is the fact that he has demonstrated at least three different immunologic types or strains with immunologic differences among the pigeon strains isolated If I remember correctly the previous studies of that nature have pretty well thrown all the pigeon strains together At least that is true with the toxin antitoxin tests and few people have done cross immunization with a whole series of pigeon strains as is done here

It seems to me that one of the approaches to the general aspects of the psittacosis problem is to determine to what extent strains are passed back and forth among the various hosts that we know about and immunologic labeling may be one means of determining whether or not such transfer occurs Of course the question of whether these agents change in character with passage from one host to another has to be answered. But with respect to

cross immunization I should like to ask about the technic employed

DR SHAUGHNESSY We used live virus to immunize groups of mice which were then injected with decimal dilutions at the same time normal controls were injected Then we took the end points in which we got the protection in each instance as shown by the results in the normals and compiled the results I neglected to point out that we didn t show the controls in the table here in order to conserve space but the results were fairly sharp in these controls

DR WENNER I should like to comment briefly on Dr Goss s point because it seems to me that we can use two indices here One is the clinical index of infection and it seems to me that in New York City for instance there must be very little known at the moment about respiratory infections directly traceable to pigeon lofts The other is the laboratory index which if so highly positive would indicate a great deal of inapparent mild respiratory infections

DR SABIN There is a study of an epidemic in Cincinnati which throws some light on this question In 1947 we were faced with an outbreak that was called pigeon pneumonitis which involved some men who had cleaned an old abandoned water tower that was full of tons of pigeon excreta and which was inhabited by pigeons

Twelve men developed pneumonitis after an incubation period of seven to 14 days and we thought that we should have no trouble proving that it was psittacosis Well the pneumonitis was not caused by ornithosis–psittacosis virus But of 24 pigeons trapped in the tower about 50 per cent had complement fixing antibodies for psittacosis It was not until several years later that we discovered that the cause of the pneumonitis was *Histoplasma capsulatum*

I think the moral of this story is that we must learn more about the virulence of the viruses that are present in these pigeons Perhaps it would be wise to dispense with the testing of organs of pigeons and concentrate on the droppings to find out something about the quantitative aspects of virus carriage

We all know that a dysentery carrier doesn t necessarily mean

a dysentery spreader Here I am invading the territory of Dr Shaughnessy A person who has a few *Shigella* in his stool isn t very important but a person who has millions is very important.

Now am I right in saying that we know nothing of the sort about pigeons in relation to parakeets and parrots? The pigeon may put out one infectious dose in his droppings the parakeet a million

DR SHAUGHNESSY I think Dr Sabin s remarks are very pertinent and I should apologize by saying that these are the results of rather old studies of ours We abandoned this phase of the work to investigate other aspects of psittacosis

But in retrospect it seems to me that we should have studied the incidence of psittacosis virus in pigeon droppings It seems to me that someone should begin to see whether the viruses can be collected in the air in sufficient concentration to be possibly infectious around some of the places where pigeons abound

I m glad Dr Sabin brought up that outbreak as I thought that it was an outbreak of psittacosis when I saw a reference to it and so I looked it up Of course the clinical picture was quite different in that outbreak compared with the one that we described in which we had a fairly mild fairly transient disease Dr Sabin s was a typical histoplasmosis outbreak clinically that is with a very long course

DR SABIN The clinical spectrum can mislead you because some people were sick for three days and others for 19 Not until the serologic data were obtained did we know what we were working with

DR POLLARD I wonder if perhaps we are studying the wrong aspect of this disease in pigeons Instead of studying the egress of this virus we should study the virus itself and find out why some strains are less virulent supposedly than those which are found in humans associated with parakeets or with other species of birds Perhaps the virus in pigeons is less toxigenic and therefore less pathogenic than the virus in turkeys or in the psittacine species

DR. SHAUGHNESSY That is one reason I mentioned that it appeared that we may have isolated viruses of the Illinois type because that is a rather virulent or toxigenic strain The fact that

a laboratory worker was infected with it and that we isolated it originally from two fatal cases would indicate that wild pigeons do not always carry viruses of low virulence or toxigenicity

DR DAVIS It seems to me that a great deal more information about the disease in man caused by viruses of pigeon origin is needed We need a lot more information, too about these localized outbreaks originating from pigeons—the kind of information that Dr Irons obtained from his studies in turkeys and that Dr Korns obtained from his studies of duck psittacosis

Actually, most of our information about pigeon psittacosis comes from isolated cases with the exception of the outbreaks which Dr Shaughnessy has described and we are probably just picking off the more spectacular cases which come to the attention of the physician

It has been pointed out that when this disease was looked for in hospitals and in clinical practice it was found in a varying proportion of the cases It is probably true that many many cases are missed simply because the physician does not associate the disease with the bird or fails to get a serologic test It leads us to wonder of course as has been pointed out whether there are not a great many variations in the severity of the disease There may be a great deal of inapparent infection or infection of a very low pathogenicity So it is certainly in order to continue some very careful controlled epidemiologic investigations on these diseases

Now just a word about the serologic diagnosis We of course realize that it is important to show a rise in antibody titer and that a single test probably doesn t mean very much

I recall a few years ago seeing a patient a sailor who had X ray evidence of a pneumonitis When we tested his serum he had a rather low titer 1 16 or 1 32 and we thought we had the early stages of psittacosis At the end of the war he had actually brought back some psittacine birds from South America When we took a later sample, the titer was just the same or perhaps a little bit lower and we failed to isolate any virus On reviewing the history more carefully we found that he had had a lymphogranuloma venereum infection a year or two before which might easily have misled us in this case

CHAIRMAN STAFSETH Dr Morris Pollard Professor of

Preventive Medicine and Public Health University of Texas Medical Branch Galveston will now present a paper on "Psittacosis in Seashore Birds"

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CHAIRMAN STAFSETH Dr Leonard J Goss Assistant Director veterinarian New York Zoological Society New York City will now present a paper on "Psittacosis in Zoological Collections"

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DR. SUSSMAN In the records of the Washington Zoo was there any reference made to the previous occupation or avocation of the man who was there for only 35 days before he came down with psittacosis?

DR. GOSS He apparently had not had any other avian exposure

DELEGATE You said that the parrot you autopsied showed some lesions What were the characteristics of these? Was there an enlarged spleen or a necrosis of the liver or other changes?

DR. GOSS No there was no visible gross necrosis of the liver The spleen showed an acute splenitis There was seromucous rhinitis congestion of the lungs and opaqueness of the air sac membranes We did not observe anything in the pericardium.

DR DAVIS I just want to follow up on that information about the experience at the Washington Zoo When I started work on psittacosis in 1945 I tested the serum of one patient for complement fixation and at that time he did have a titer that was fairly significant so I dont think there is any question that he had psittacosis

I should like to ask in reference to the tests on bird serum which you reported as tested by the *complement fixation test* was that the direct complement fixation test or the indirect?

DR GOSS Direct

DR DAVIS I certainly feel that when we have a larger experience with the new techniques for example the indirect complement fixation test we will have a lot more confidence in the serologic study of bird serum—than I do at least at the present time with the older methods

DR. SABIN While we are on technical questions I was in

trigued by your statement that you used heparin when you bled birds. Now, heparin is known to be anticomplementary, and I was wondering whether the laboratory reported having trouble, or is a dilution of one to eight enough to get rid of the anticomplementary effects of heparin?

DR COSS I wondered about that Dr Sabin and as I said, these samples were sent to Dr Meyer I inquired about heparin and he said he didn't care that it didn't make any difference what we used As I stated in the paper in collecting some of the samples we used heparin and in some we used chemical anticoagulants.

DR COTTRAL There is a recent article, in 1952, by Wilson and Matheson in England about bird migration and foot-and-mouth disease They by statistical studies, have come to the conclusion that in England at least many of the outbreaks may be traced to birds migrating from Continental Europe to England I just put that out for additional information relating to birds as disease vectors.

DR HUEBNER From the technical standpoint it seemed to me that given the high rates of infection that are demonstrable in certain species of birds, and the high rates of complement fixing reactions that are observed in these birds, it should be possible to do an evaluation of the various available immunologic procedures in terms of demonstrable infection It is surprising with all the amount of work that has been done with respect to actual use of these methods and techniques that someone has not taken the trouble to study a large population of birds with a high prospective rate of infection and do virus isolations, under careful conditions to make sure there are no spontaneous or accidental infections, and to relate the results to the various serologic methods that are available It seems the obvious thing to do because certainly one cannot rely on any serologic method to give wholly reliable evidence of infection particularly if one wants to find out what it means in terms of demonstrable infection In relating our serologic experiences with those of other laboratories it has become obvious to us that the most necessary thing often is an objective evaluation of tools so that we know what they mean Why

do we go on running these tests ad infinitum without finding out what they do mean It can be done

We were able to do that for instance with Q fever in Los Angeles We used a very similar test (complement fixation) to that used in psittacosis We took groups of 400 or 500 serums and shifted them around to various laboratories and observed that different techniques often gave quite different results

We have had the same experiences in our own laboratory Changes in techniques increasing sensitivity or increasing specificity have made all the difference in the world between finding 20 or 30 per cent of our animals nonspecifically positive in areas where the disease does not occur and finding them altogether negative We discovered however that with respect to Q fever our test could be evaluated It was possible to do this for cattle by performing fairly large scale serologic surveys plus virus isolation attempts in a number of infected herds Specimens were taken on the same day and it was possible then to relate any serologic test to the presence of the microbe Thus we were able to predict on the basis of the level in a test how many quarters of a cow would be infected We were finally able to select 40 cows with a titer of 1 32 or higher for a therapeutic study every one was shown to have multiple infected quarters I should think that similar evaluation might be done here with respect to each species of birds and with each test

DR POLLARD I think that Dr Huebners remarks are well taken A word of explanation might come in here

Much of this work was done at a time when these species of birds were unexplored and the incidence of the disease among them was unknown So far as I know every species of birds that has been extensively investigated has been positive but when one does find positive serologies correlated with positive isolations among them they have served their purpose Certainly we should always attempt to make our techniques more sensitive than they are There has been fairly good correlation between positive serologic response in birds and positive isolations As far as I know there have not been positive serologic reactions and negative isolations

DR HUEBNER I am sure that this correlation exists there is no question about that But the real issue is this when we test for instance, a flock of birds or a herd of cattle and by one test find 50 per cent positive and by another test 10 per cent positive we may still have a correlaton but we also have a problem on our hands

DR STEELE We have recognized that problem at the Communicable Disease Center, and we hope to set up a project this coming summer We have been approached by various bird associations and budgerigar societies that are interested in cleaning up their industry The thing that we have reservations about is can we use the tools that we have at hand and until those tools are tested can we offer these organizations the assistance that they need

DR HUEBNER There is the distinct possibility of using epizootiologic methods to test immunologic tools It requires significant numbers and a comparison of one method of testing to another I think it can be done

DR SHAUGHNESSY I should like to bring out another aspect of this problem that we encountered recently in connection with a case of human disease

We used to make our own antigens but recently we have been purchasing them except those used for research purposes and one manufacturer we discovered later (we didn t know it at the time) was putting out an antigen consisting only of the soluble substance

Now we had good titers with this in the acute phase but they faded off and we would get nothing later When we discovered that this antigen contained only the soluble substance we went back to some of those serums we had kept preserved and tested them with the whole antigen and we found very great rises in titer

In other words we have called a number of specimens negative based wholly on findings with a bad antigen so in analyzing our data I think we have to keep factors of that nature in mind also

CHAIRMAN STAFSETH Dr W L Treuting Professor of Public Health Administration Department of Tropical Medicine and Public Health The Tulane University of Louisiana School

of Medicine will now present a paper on the "Epidemiology of Louisiana Pneumonitis"

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DR KISSLING We have very good evidence that there was human contact infections in the Louisiana epidemic The source of the original infection was rather obscure This area is one in which the trapping was mainly for muskrats and nutria but when these two species of animals were examined for susceptibility to the infection it was found that they were refractory at least to the clinical disease However since they had been collected in that area they may have recovered from the disease and therefore showed a certain amount of resistance

In 1950 as Dr Treuting said while we were surveying for encephalomyelitis virus in birds in Louisiana we isolated a psittacosis virus from snowy egrets in southeastern Louisiana on an island in Barataria Bay These egrets as you may know nest in very large colonies There are rookeries where several thousand birds nest together They may have anywhere from 15 to 20 nests in one small bush or tree and there is very close contact among these birds during the nesting season Also there are several species that nest together the snowy egret American egret, and other herons all nest at the same time in close proximity

We did notice that some of the young birds were rather weak and emaciated at the time we were bleeding them We did not take into account and mark down the particular bloods from the sick birds We assumed because of the many natural deaths that there are in wild birds that perhaps the adults had been killed in some way or another

During the nesting season these rookeries are invaded by the human population to take birds for food The birds are considered quite good although they smell rather fishy and are not very appetizing when you go into the rookeries But we do know that these birds are used for food occasionally and though people will admit off the-record that they take these supposedly protected birds officially they don t want to admit it

The virus that we isolated was pathogenic for mice by intracerebral intraperitoneal subcutaneous and intramuscular routes It was highly virulent for guinea pigs by the intracerebral and in

traperitoneal routes Guinea pigs that were immunized against the Louisiana pneumonitis strain of virus resisted infection with the new agent isolated Guinea pigs immunized with the 6 BC strain of psittacosis virus were fully susceptible to the new strain

Our neutralization tests were not very conclusive By the in tranasal neutralization test we could show no essential difference between the pigeon psittacine or pneumonitis strains and the new agent isolated However, there was some indication that toxin neutralization of the new strain did occur with the Louisiana pneu monitis antiserum Insofar as we can tell it is at least quite similar to the Louisiana pneumonitis virus

Again in the following year we went to an area around Bunkie which is in the east central part of the state and collected some birds to take back to the laboratory for encephalitis work This rookery was again one of American and snowy egrets and about a month after the birds had been in captivity we noticed that one American egret became atoxic and that it quickly progressed to prostration and death Within a week we observed the start of a severe epidemic in our captive birds which within a month s time wiped out the entire flock

These birds were emaciated on autopsy the spleens were en larged and there was a fibrinous peritonitis and pericarditis Again a virus belonging to the psittacosis group was isolated and it showed the same pathogenicity for mice as the previously isolated strains

We cannot rule out the possibility that these birds were in fected after they were brought to the laboratory since psittacosis virus was being used in the laboratory However the aviary where the birds were kept was at a considerable distance from the labora tory where psittacosis virus was being used The caretakers re sponsible for these birds had no contact with the experimental animals in the psittacosis project Thus there is some evidence that the Louisiana pneumonitis virus does have a reservoir in wild life

May I ask Dr Irons whether or not the turkey psittacosis virus has been typed

DR IRONS I do not know that Dr Meyer has typed it Perhaps Dr Pollard has been attempting to do so We have not but all the

information we have strongly suggests a close relation between these two viruses

DR STEELE Dr Meyer told us at El Paso that these are two different viruses and the only similarity is their toxigenic properties

DR IRONS I am glad to have that information because the viruses certainly do have some properties—superficially at any rate—which are very similar Maybe he has used criteria that prove that they are different viruses Both certainly are pathogenic for guinea pigs and both are very toxic He told me the turkey virus is as toxic as any virus that he has ever encountered He wasn t at all surprised to learn how sick some of the patients became or that there were deaths he was only surprised that there weren t a great many more deaths than there were Despite the evidence of neglect of some of the patients (some got treat ment very late) he thought that after all the physicians must have had pretty good luck with their patients in general

There are several interesting things about these epidemics Of course we were aware of the virulence of the agent when we were studying the turkey psittacosis outbreaks and as we saw more of the patients we warned those around the hospital the doctors and nurses how dangerous these cases might be How ever we also took into consideration that since they were on antibiotic therapy maybe they wouldn t be quite so dangerous

At any rate we had practically no evidence of transmission from one person to another In my description of these last out breaks perhaps I should have said there was a Mexican clean up man who became ill several days before the rest of them The question might have arisen as to whether this person could have been the source of infection for the other persons who became ill later But the evidence would not support that because this Mexican had a large family and there was no illness among the members of his own family and yet there were at least eight other illnesses which developed around Christmas And the incubation period would have been much too long anyway

The question was raised repeatedly in regard to these later out breaks at Giddings as to whether the infection in some of the

cases couldn t be traced back to some carrier who had returned to work rather than to the turkeys Well, I think there are all kinds of evidence—there was the same series of circumstances and there was no evidence that any household contacts ever acquired this type of infection, either at the time of exposure to acute illness or later on

There was one possible exception—a Mexican woman in the second outbreak, whose daughter became ill within the hypothetical incubation period but we thought that the evidence was much stronger that the mother and daughter both were exposed probably at the same time and that in one the virus had a short incubation period and in the other, a long incubation period So altogether there was no evidence at all of person to person transmission

Now the other incident that I mentioned earlier as occurring in 1937 1938 was very interesting There was, I think a saving factor in that one physician saw all the patients and he thought that he was dealing with the old 1918 influenza and then if not that the next thing he thought of was the bare possibility of plague

He never thought of psittacosis The patients who were dying or were very sick went to one hospital and the pathologist there never thought of psittacosis either Now, I don t know whether the precautions that were taken in the hospital were sufficient to protect the physicians and nurses but at any rate of the seven people who acquired infection—and four of them died—apparently all acquired the infection from this one farm boy What the source of his infection was I cannot be sure I am of course not at all sure it was a calf—it could have been any number of other sources—but I feel relatively certain it was not a parakeet

But the point was that in the Texas case there was no transmission from any of these people in the hospital It was not a person to person spread like that described by Dr Treuting in which seven people got it from one another Whether it was the fact that in the Texas case one doctor had all the patients and practically all these went to one hospital where precautions were taken to preclude further transfers—well it is interesting to speculate on that at any rate

DR TREUTING There is an interesting note with regard to

Case 19 She is a registered nurse and was a close personal friend of Cases 17 and 18 who also were registered nurses She volunteered to nurse them and was confined to the house in which they were treated during the period of their illness The usual isolation techniques were used She along with all other attendants was furnished with the type of mask described by Dr Wu Lien Teh for use in pneumonic plague These masks made of several layers of gauze with cotton in between completely covered the lower half of the face with cotton plugs inserted along the sides of the nose Though uncomfortable all attendants wore them when with the patients In spite of such precautions she developed the disease while in quarantine following the deaths of Cases 17 and 18 (The quarantine period was set at 21 days—the longest possible incubation period according to our calculation)

When Case 19 became sick we were faced with the problem of nursing care for her Only one nurse in the area who had also attended Cases 17 and 18 volunteered It was necessary to get two volunteer nurses from New Orleans They remained in attendance during her illness living in the house in which she was confined

Case 19 was very sick her illness during its early stages paralleling that of fatal cases Her recovery may have been due to the transfusion of whole blood and of immune plasma from previously recovered cases in the series Sulfonamides were ineffective in all cases in which they were tried

DR. SHAUGHNESSY I think that you might be interested in a case of person to-person transfer that came to our attention during this past year Because of an extreme shortage of beds in a hospital, a surgical patient and a patient with pneumonia were put in the same room It was assumed at that time that the patient with the pneumonia had a bacterial pneumonia All possible precautions were taken to prevent transfer of infection from one patient to the other and in addition to that the surgical patient was given penicillin for four days and then was given large doses of aureomycin for several days after that But in spite of everything the patient developed psittacosis and both of them were shown to have psittacosis by rising titers in their complement fixation tests

DR HUEBNER You mentioned that no children came down with the disease but how many children were present in the households and to what extent might they have been exposed during the incubation periods of these cases perhaps very in tensively?

DR TREUTING Children were present in many of the homes in which cases occurred but I do not remember the number I recall two or three in the household in which Cases 17 and 18 occurred They remained in the house throughout the illness of these cases I should think that children in the households were in intimate familial contact with cases during the incubation period but the extent of exposure might be difficult to measure

Whatever contact the children had with the cases during their illness would have been relatively casual There would not have been the close nursing type of contact especially late in the dis ease which seemed to be essential to its transmission

DR HUEBNER Well is it true in psittacosis as it is in rick ettsial diseases and some of the virus diseases that the younger age groups are somewhat less likely to develop the clinical dis ease although they are equally susceptible to the infection? It has been generally stated here that psittacosis must be much more widespread than realized—that perhaps infection is much more widespread than the frank illnesses would indicate even if the lat ter were all recognized If it is true that children are susceptible to infection only such a fact would have a considerable effect upon the epidemiologic pattern of the disease in the human host It may be that a great many of the younger people were im munized during your outbreak and that age and dosage might have been the important factors which determined what hap pened to those members of the household and community who did become ill I think what is most needed in the whole problem of psittacosis is a more accurate idea of the spectrum of infection

DR TREUTING Certainly this may be a widespread infec tion with relatively little disease occurring In the episode de scribed there were many people exposed who did not become ill The reason may be something other than the type of exposure This might be a commonly occurring infection in the population

of that area To my knowledge no means are presently available to test for this

DR HUEBNER Can anyone answer the question of age susceptibility to the disease?

DR DAVIS I cannot answer that completely but I think you will find in the literature that about the youngest case of psittacosis which has been actually diagnosed is nine years old As the age group increases—on the basis of the meager experience that we have and granting the fact that we do not see all the cases of infection—the specific attack rate increases up to about forty years of age when it remains fairly uniform and also that in the period before antibiotics the older people suffered a greater risk of fatality

I should not think that immunizing infections played very much of a role in the occurrence of the disease There is a good deal of evidence to indicate that the immunity as produced by this disease is really only partial and I think Dr Irons showed yesterday that there were actually cases in these plants of individuals who had had a previous infection There have been in the literature at least two recorded cases of second infections occurring at a considerable time later The evidence is also borne out by the experiments in animals in which it is very difficult to get a good high solid immunity I think we are dealing with a somewhat different kind of immunity in the psittacosis group than exists with some of the other viruses

DR WITTE Is there any evidence to show that some of the recovered cases continued to shed virus?

DR TREUTING There is no evidence on that Nothing as far as I know has been done to follow up the cases Several hundred specimens of blood were collected from contacts and from recovered cases for serologic study and I think Dr Olson still has them

DR POLLARD There is a record in the literature by Dr Meyer reporting on one person who years later I think eight or 12 years still had infective saliva This may be exceptional but the answer is that it is possible

DR IRONS Regarding the insistence on the part of the poultry plant people that they were being unjustly accused I may say that

we gave serious thought to the possibility of human carriers and so on Dr Meyer s suggestion, we selected those people who seemed to have titers persisting at unusually high levels

We not only took throat washings from them but we also took urine samples and concentrated the urine samples to a consider able extent and did quite a bit of testing We did several tests on two or three people and at least a single test on at least eight or ten but not from a single person did we succeed in recovering a virus from throat washings or urine samples

DR WENNER We have tested about 100 children over the last three years against a battery of antigens to try to delineate their respiratory infections The serums were tested against the psitta cosis antigen We found none that had antibodies against psitta cosis We found one serum that reacted with Q fever antigen a titer of about 1 32 a rise of about fourfold We do not know the causative agent in some of the peculiar bronchial pneumonias and bronchiolitis infections that occur in children

DR POLLARD If there is a possibility that psittacosis is a mild disease in childhood could it be possible that we are misinterpret ing the importance of lymphogranuloma venereum then in at tributing the high incidence of positive serologies among Negroes to LGV rather than psittacosis and conversely, if we are why is it confined to Negroes? If it is a respiratory illness why is it not community wide?

DR STEELE Dr Parks will probably take it up in the next paper with reference to some of Dr Stoenner s work in Florida in which they surveyed employees in poultry plants throughout the state There was a fair number of persons with complement fixing antibodies

Then they ran similar tests in Salt Lake City an area which is thought to have a low incidence of LGV but the same thing turned up out there Now he is trying to explain the high level of complement fixing antibodies in these two communities

CHAIRMAN STAFSETH Dr L L Parks Director of the Bureau of Preventable Diseases Florida State Board of Health Jacksonville will now present a paper Report on the Psittacosis Problem in Florida "

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Afternoon Session, Second Day

Dr J O Dean Assistant Surgeon General Associate Chief Bureau of State Services United States Public Health Service Washington D C presiding

DR IRONS The psittacosis problem and the practical side of just what to do about it as well as just how important it is in the way of a total public health problem is one that has aroused a lot of curiosity in Texas because we like Florida and California raise a lot of birds of various kinds and some turkeys that go out of the state It was conservatively estimated a few years ago when the interstate regulations went into effect that there were 7 500 people who were raising parakeets I am told now that that figure has probably been doubled or tripled and some people are raising enormous numbers of birds I should like for Dr Young or anybody from the Ohio State Health Department to comment on studies that are being made now there has been some correspondence between us regarding psittacosis in Railway Express workers I do not know whether that concerns parakeets in particular or all birds coming from Texas but I believe Dr Young Public Health Veterinarian State Health Department of Texas has seen some of this correspondence and perhaps he may want to comment on it

DR YOUNG The correspondence concerning the Railway Express workers in Ohio was received just before Dr Irons and I departed to attend this meeting The two cases mentioned involved exposure to parakeets originating in Texas Both cases showed a marked rise in titer following the infection

Two points that might be discussed at this meeting are one the desirability of requiring bird sellers to keep sales and purchase records and second how extensive should our investigations of non psittacosis clinical illness be in aviaries In reference to point one leg banding for a permanent record of the bird should be considered

Psittacosis in psittacine birds does not seem at present to be much of a public health problem in Texas in that we agree almost exactly with what Dr Parks reports from Florida

Our present approach to the public health aspects of psittacosis is to check back from human cases to the aviaries to see if there are sick birds there showing psittacosis and shedding the organisms The mere presence of psittacosis titer does not warrant action as we see it because we believe there are few if any, psittacosis free flocks

The situation does not seem to justify any regulatory action and the same is the case in Florida We are not contemplating any In brief the two things that we are currently concerned with are whether to require record keeping and how extensive our investigations should be keeping in mind the limits of time and service to be properly apportioned to our whole disease spectrum

DR PARKS I agree with Dr Young that we should keep a record or ask the sellers to keep a record of where the birds come from and what disposition is made of them Again I am not too sure that it is practical If we have as many bird dealers as we think we have in the State of Florida I am sure that we do not have sufficient staff to supervise the job properly It would be worth while to ask the dealers to keep records and leave the burden upon them to answer the question if they should have trouble in their farms

I think there are many unsolved bird problems in our state as well as other health problems that could be investigated if we had the time and personnel I should like to make additional studies on the bird handlers as well as carry out more sampling of birds to determine if we do have a problem

Yet on the other hand our services have to be limited to certain fields and we must draw a line somewhere Unless we have more trouble than we think we are having now I do not believe that it is advisable to spend more time on psittacosis—unless something develops that we do not foresee

DR POLLARD There was expressed an opinion in the last paper that the complement fixation tests for this disease were not very good and that people who had syphilis gave positive tests for psittacosis I think that this one fact should be called to mind if the test antigen happens to be Lygranum the directions state specifically that it should not be used with luetic serum Moreover if we run this test on luetic serum we should use either an extracted

antigen or an antigen prepared from chorioallantoic fluid and then test it as to specificity with luetic serum By running these tests and merely saying that on the basis of these conditions the test was either inaccurate or no good I think we are assuming more than the results would warrant

DR HILLEMAN In discussing the specificity of Lygranum in tests with syphilitic serums one must define what one means by Lygranum This material has been made by different formulas over the years When first prepared the antigen consisted of the high speed sediment of a suspension of yolk sac infected with the virus of lymphogranuloma venereum This material was relatively crude and the yolk or volk sac substance which was present reacted with the serums from patients with syphilis The specificity of the test result was controlled of course by simultaneous serum titration with normal volk sac antigen

Around 1945 the phenol-enhanced antigen was introduced In its preparation the syphilis reactive antigen appeared to be largely destroyed so that it no longer reacted significantly with serums from patients with syphilis In addition the virus antigen was present in such high titer that the preparation was used in a dilution (about 1 1600 of yolk sac) which contained negligible amounts of yolk sac material

DR. IRONS In a paper which we wrote we mentioned that very point that we had no trouble with syphilitic serum reacting nonspecifically with this antigen

DR HILLEMAN May I add to Dr Irons remark that we have used the phenol enhanced antigen at the Army Medical Service Graduate School and in the Army Field Laboratories since 1948 This antigen proved highly satisfactory for detecting antibody in serums of patients infected with viruses of the psittacosis lymphogranuloma venereum group and did not react nonspecifically with serums from patients with syphilis It also is important to remember in this connection that infection with viruses of this group has a strong tendency toward latency and that antibodies may persist for many years following a clinical or subclinical infection When testing syphilitic serums one is for the most part working with specimens from individuals who have been venereally exposed and in whom the chance for past lymphogranuloma venereum in

fection is great For this reason, a good many serums which are positive for syphilis are likewise positive for lymphogranuloma venereum

DR WITTE I have one question to ask of Dr Parks Have you gotten any reports from the Montgomery Laboratory on those additional parakeets? I am from Pennsylvania and we are anxious to know what has turned up

DR PARKS We sent in bird specimens, but I have not received a laboratory report They had no record of selling the birds but I understand that further investigations have been made with the information that they have had some ill birds, but I have no laboratory report

CHAIRMAN DEAN Dr Calvin B Spencer Medical Director and Chief Division of Foreign Quarantine United States Public Health Service Washington D C will now present a paper on 'The Development of United States Foreign Quarantine Regulations for the Control of Psittacosis since 1930

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CHAIRMAN DEAN Dr James H Steele Chief Veterinary Public Health Communicable Disease Center, United States Public Health Service Atlanta will now present a discussion paper

DR STEELE The review which Dr Spencer has presented covers the history of the quarantine control of psittacosis in the United States It is interesting to note that the quarantine regulations were revised quite often during this period and made enforcement difficult The successful treatment of psittacosis with broad-spectrum antibiotics probably more than any other single development influenced the changed attitude of public health authorities The increased knowledge of the wide host range of the psittacosis lymphogranuloma venereum group of organisms altered previous concepts that quarantine of any one kind of animal could control psittacosis Another factor which was considered was the cost of administration in light of the importance of the problem The hope that research could eventually provide more satisfactory control procedures through immunization prophylactic therapy and environmental hygiene was also expressed by some authorities

The addition of new observations to the world literature has revised drastically the concepts of the epidemiology and epizootiology of psittacosis in the postwar period Previously the infection in man had been associated mainly with caged birds and investigations were centered on this reservoir Shortly thereafter, studies revealed that canaries finches thrushes and doves also are infected Following these observations there was a series of reports of spontaneous psittacosis or ornithosis in sea birds (fulmars and sea gulls) barnyard fowl (pigeons chickens ducks and turkeys) and various wild birds including pheasants egrets and sparrows

Growing in significance are isolations from mammals of agents that have all the morphological characteristics of those of the psittacosis viruses They are found in the respiratory tract of apparently healthy or diseased mice cats sheep and goats in the intestinal tract spleen and brain tissue of cattle and in the placenta of aborting ewes

Natural infection has been proved in at least 72 species of birds of ten orders and 52 genera Two common characteristics of all these species are the acute fatal disease it causes in young birds and the high incidence of prolonged latent infection in all age groups

Psittacine birds including parrots and parakeets all over the world can be reasonably suspected as reservoirs Severe widespread epizootics have been reported among wild parrots and rosellas in Australia and Tasmania parakeets in South America and gray parrots of Portuguese East Africa Epizootic phenomena such as those seen occasionally in wild psittacine birds have not been reported in captive birds

The type of infection which occurs most often in caged birds is an inapparent infection producing no gross signs or symptoms beyond an enlarged spleen Many birds probably become infected in the nests and remain immune to reinfection Sudden changes in environmental conditions low temperatures crowding improper diet and dampness may weaken the host favor the parasite and induce relapses Virus dispersed in large quantities in the atmosphere of crowded quarters spreads the infection rapidly from diseased to susceptible birds Some die but a great number be

come carriers Under such conditions it is not surprising that birds apparently healthy when shipped are suffering from psittacosis by the time they reach their ultimate purchasers

According to Meyer, the incidence of carrier infection may reach 50 per cent among psittacines It is difficult to find commercial aviaries free of the disease The incidence of latent infection varies from 10 to 80 per cent in psittacines

Canaries and finches are thought to become infected following exposure to diseased psittacines However naturally occurring disease has been reported in canaries

Evidence of psittacosis among domestic and wild pigeons was first recorded in 1940 The infection rate ranks high among the non psittacine birds ranging from 5 to 75 per cent in flocks Doves and bleeding heart doves show high infection rates and latent psittacosis is not uncommon among them

Chickens have a low grade type of latent psittacosis which causes no acute or relapsing disease and can be recognized by the complement fixation inhibition test The percentage of positive chickens varies from 24 to 50 per cent according to available data There are no reports of epizootics of psittacosis among chickens

About 40 per cent of the ducks tested by complement fixation inhibition tests give positive reactions The infection is of an inapparent type and no epizootics of psittacosis have been reported Young ducklings are thought to become infected in hatcheries this is supported by the isolation of the virus from the liver spleen and kidney of ducklings which died in a hatchery

A highly virulent psittacosis virus which has been isolated from turkeys in recent years is similar to the egret and Louisiana pneumonitis type Meyer reports that the complement fixation inhibition test revealed infection rates from 6 to 38 per cent There have been five epidemics of occupational psittacosis attributed to turkeys since 1949 More than 150 persons were reported stricken and seven died In addition relapses and reinfections were recorded * The disease in turkeys is considered enzootic with occasional epizootics resulting in some fatalities Diseased birds

* In five additional outbreaks reported in the spring of 1954 by J V Irons about 200 persons were ill.

become emaciated and show respiratory symptoms the spleen is grossly enlarged egg production falls off rapidly and hatchability is drastically reduced Attempts are now being made to discover if transovarian transmission may take place Studies are also under way to determine if the virus may survive in the carcass and in the environment after processing

Pheasant serums examined by the complement fixation inhibition test were found to be positive in significantly high titers Human cases have been reported on game farms where sero positive pheasants were found. Psittacosis virus has been found in snowy egrets and fulmar petrels

Investigations during the past few years have established the presence of psittacosis in at least four mammalian orders rodents carnivores marsupials and ungulates The virus has been isolated frequently from the respiratory tract of apparently healthy mice Hamsters and guinea pigs are also susceptible to the psittacosis viruses

Meyer is of the opinion that feline infections are widespread and there may be feline carriers Feline pneumonitis is a common clinical entity but the etiologic agent is rarely identified On the basis of the available information the disease may be considered enzootic among cats although the disease is described as highly contagious there are no reports of epizootics among them

An agent described as a psittacosis virus has been isolated in calves and adult cattle and it is apparently widely distributed in certain herds The bovine encephalomyelitis virus (BEV) is one of the psittacosis virus group It is important to note that the bovine viruses isolated do not infect mice but guinea pigs are readily infected by the peritoneal route Meyer reports that BEV has produced human illness in a laboratory worker

Psittacosis virus has been reported as the cause of abortion in sheep in Scotland A recent report from Japan by T Omari *et al* describes the isolation of the causal agent of caprine epizootic pneumonia The virus is related to members of the psittacosis group The disease was reproduced readily in goats Viral agents of the psittacosis group also have been recovered from the brain of Columbian opossums which were showing paralytic symptoms

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DR DAVIS Perhaps one of the reasons for the increased number of reported cases of psittacosis is the effort on the part of the National Office of Vital Statistics to report outbreaks not only of psittacosis but also of all other infectious diseases This started about a year and a half ago and through this method the attention of many public health officers and physicians has been brought to the fact that psittacosis is occurring and consequently they are more aware of the danger of the disease

We must realize that these reported cases are for the most part "selected cases recognized because of the astuteness of the physician or the hospital either in noting that there was contact with a bird or in requesting a serologic test

CHAIRMAN DEAN There certainly has been more attention to reporting of the new diseases How conscious the states are I do not know but in the United States Public Health Service we are at least conscious of attempting to encourage a more extensive reporting

DR STEELE As Dr Spencer pointed out in his paper in 1953 there were 73 cases of psittacosis officially reported to the National Office of Vital Statistics but through the efforts of Dr Martin Hicklind the medical officer who was responsible for keeping track of the human cases at the Communicable Disease Center and Dr Scruggs who keeps track of the bird outbreaks they have been able to uncover through private correspondence records of another 100 cases that had not been reported through any existing channels

DR SUSSMAN I think those 100 cases suggest the possibility that some state officials neglect to report some cases of psittacosis either in birds or in humans on the assumption that to do so might hamper an industry within that state

This is particularly true if a good laboratory is available in a state enabling the investigator to find psittacosis if he looks for it but if there is no such laboratory the disease cannot actually be diagnosed It still is not clear in my mind whether if we reported all such cases New Jersey itself would not be quarantined Maybe Dr Spencer could answer that

DR SPENCER In trying to answer that specific question first let me say that that is just a bit out of my sphere of activity since

The pathogenicity of mammalian strains of the virus for man has not been conclusively proved but the constant occurrence of specific psittacosis complement fixation reaction in persons in close contact with sheep and cattle suggests that latent infection with mammalian strains is a possibility The public health aspects should be studied

No doubt further investigations will find the psittacosis lymphogranuloma agents in numerous other avian and mammalian hosts There are few other diseases which have such a wide host spectrum

The increase of human illness from this virus in recent years points to the importance of psittacosis as an occupational disease among poultry plant employees Dr Irons has reviewed this problem very thoroughly and is to be commended for his investigation Another problem on which little has been reported is the prevalence of psittacosis in the squab industry Psittacosis is a common disease among squabs and pigeons but there is little or no information on the occupational disease implications Most of the human cases reported in which pigeons are suspected as the cause of disease are usually among fanciers and racing pigeon enthusiasts A recent outbreak of 17 human cases on a foreign military reservation was attributed to exposure to pigeon cage droppings and dust Interest in cat scratch fever (benign lymphoreticulosis) has raised the question of whether or not it has any relation to psittacosis To date there is no information which might show such a relationship

It is well to emphasize that everyone who is concerned with the control of psittacosis has an important responsibility in solving this problem The pet bird industry may be expected to support research to provide the answer to control among parrots parakeets finches and canaries The domestic poultry raisers have both an economic and occupational disease problem to resolve The significance of mammalian psittacosis is not fully understood but those who have reported on this disease point out that it may be of serious consequence under certain conditions

There is also need for comprehensive studies of bird environmental factors including breeding management nutrition and population densities and their effect on disease

DR HOLDEN Following my part in the investigation in the Florida outbreak I submitted an unsolicited recommendation to the Communicable Disease Center with regard to suggested changes in the foreign quarantine regulations for the importation of psittacine birds At this time I recommended that we permit the importation of psittacine birds to be sold by dealers to potential owners as well as to exhibitors and research workers My reasoning was partly in recognition of the popularity of these birds in the United States

By virtue of the restrictions on the importation of birds the demand has greatly exceeded the supply with the result that prices have gone up and smuggling has become a profitable industry In other words I think that the restrictions are in part responsible for the industry of smuggling birds into the country I know that there was a sharp increase in smuggling during the Florida outbreak, when all import permits were suspended temporarily

I believe that the Foreign Quarantine Service should issue permits for dealers to import birds for the retail trade They could continue to isolate the birds as they are imported and have serologic tests performed and the positive birds destroyed In my opinion if sufficient birds were imported under these circumstances the price of psittacine birds would be reduced to the extent that it would no longer be profitable to smuggle birds under conditions that are most likely to introduce new and perhaps more virulent strains

CHAIRMAN DEAN You are saying that the problem carries its own seeds of correction

Dr Holden I am just suggesting that as a possibility

DR SPENCER This is one of the things that we have considered too However the licensing of regular dealers and the institution of control on the methods of handling and on the sanitation of their establishments involves a tremendous effort with a lot of personnel and therefore in trying to assure that we have gone in the proper direction and effected a control we should tremendously increase our cost operation And I m not certain that many of the large psittacine species are being smuggled Most are the smaller types

I m dealing with the foreign importations But what we are trying to do is to get a realistic view of how great this problem is but not to have it work as a boomerang on the industries within the states

There is only one way of finding out the magnitude of the problem and that is to enlist the interest of all the workers in reporting cases and the sources from which they occur after that we can intelligently proceed to do something about control if that seems indicated

Now diverging from that point may I call attention to something that was mentioned in a paper on the philosophy of approach to the controls for importation

The control of psittacosis or the importation of the birds of the psittacine family is only one small facet of our consideration or our attempted control of the introduction of contagious diseases into the country It is quite alarming from our standpoint to see the tremendous extent of psittacosis which takes in you might say a broad spectrum of animal life Then when we draw parallels epidemiologically between that and other diseases we begin to wonder if perhaps our sights are very much narrowed and that we should draw these parallels on into control on an intelligent basis if we can find that it might be possible to prevent the introduction of other diseases that can go across the same spectrum

Now bear in mind that we have receptive characteristics in this country for many diseases We also have potential arthropod vectors that have been shown capable of transmitting various other infections What I want to say here is that my total approach to this thing is that psittacosis is only one part of the problem I think that we should relate it then to all the other diseases which present similar problems and we are going to focus our attention on you who are specifically doing research (and some of it in recent years has been rather amazing) to get guidelines which will determine our future action It is only through the cooperation of you researchers and the people who are interested in these problems that we can get somewhere in trying to protect eventually not only the people of the country but the industry

DR STEELE I think that the attitude of the United States Public Health Service in dealing with the state where psittacosis is reported was very well discussed by Dr Parks this morning

It has always been my belief that within a group doing laboratory research on a common problem one of the primary prerequisites is to establish what procedures and control testing are to be applied and then keep these procedures over the whole program

It seems to me that within this group there should be enough common knowledge to permit us to arrive at a set of ground rules centering around established procedures and tests which could be adopted between groups and investigators so that the resulting data could be better correlated This could be done I believe without any fear of the individual research groups losing or sacrificing individual autonomy or independence in their work On the other hand the establishment of a few such ground rules would give the resulting data more sense and would open the way for correlating and comparing the results of Dr Irons and for instance Dr Shaughnessy in their virus isolations

Difficulties such as I have just presented have arisen many times in my own work on psittacosis If we are to conduct identifications and other research work on these strains and in this way attempt to define them it seems to me that the time has now arrived for some commonly adopted procedures for this identification At the present I am not in a position to say where we will get the leadership for such an idea However Dr Steele a few moments ago made a good suggestion which I think should be investigated further in respect to the position of the group at the Communicable Disease Center at Montgomery Alabama I should like to see the CDC group take the initiative necessary to define and I mean define in definitive terms such acceptable procedures as the complement fixation test serum neutralization test recommended isolation procedures and so on I for one should be quite willing to adopt these recommendations in subsequent work on psittacosis

For example this morning in our discussions of a paper Dr Sabin raised a question concerning the addition of anticoagulants and their influence on certain complement fixation data Immediately we have the problem of interpreting data resulting from two different serologic procedures That is data for which anticoagulants had been added to the serums and data for which no anticoagulant had been introduced

DR HOLDEN The smaller types are bred in this country

DR SPENCER And they are being smuggled in tremendous numbers

DR HOLDEN I was saying that there would be a saving if the price of these birds were reduced to the extent that it would not be profitable to chance smuggling Therefore there could *possibly be a saving with regard to enforcing border customs* regulations

DR SPENCER Well that part of it is true The amount of enforcement that is aimed at this does not entail tremendous ex pense because it goes for instance with the enforcement of other regulations This is incident to the operation itself

DR HOLDEN I was thinking too of prosecution costs and so forth which perhaps, come out of somebody else s pocket

DR SPENCER There are some other interesting things going on behind the scenes now which I think we might let run as a test pattern for a little while until we see what is going to hap pen—before we make a move such as was suggested here

This thing has been thought out and we believed that it would increase our cost so much in trying to license properly and see to it that sanitation is correct in these establishments to keep records that were necessary that at the moment it did not seem possible for us to go into it so thoroughly

DR WAGNER May I at this time introduce an idea bearing *on Dr Steele s remarks concerning control*

Each individual presenting results of his work has included careful controls in his experiments However I have not seen many instances of what one might call common controls between research groups Although there is no question as to the validity or scientific interpretation of these data I doubt whether there is within the group assembled here anyone who possesses the ability to correlate these data and arrive at a good intelligent scientific deduction Rather I would suspect that in conducting serum neutralization tests complement fixation tests inhibition tests and so on, there were as many individual procedures and techniques as there were contributors The problem that now con fronts us is one of sifting this information and attempting to arrive at an intelligent answer as to what it all means

for psittacosis In exploring what might be done we were very promptly informed that under the previous regulation there had developed a widespread illegal traffic in psittacine birds It there fore became necessary for us to consider the practicability of a psittacosis regulation in the State of Illinois

We had found in past attempts to investigate human psittacosis infections that we invariably ran into a brick wall in trying to determine the source of birds responsible for human infections In a great number of instances—I should say in the majority of instances—we found that the dealers or the persons from whom birds had been acquired had kept no records of purchases or sales so that we were unable to trace infections any farther than the bird source included in the history given by the patient

After much deliberation we accepted the revised United States Public Health Service Interstate Quarantine Regulations as a good basic structure for the control of psittacine birds and psittacosis These regulations were augmented by provision requiring that records of purchases sales or exchanges be kept by all aviaries and dealers in psittacine birds for a period of two years and be made available to public health inspection upon demand It was our feeling that with this provision and medical alertness in the recognition of human psittacosis we should be able through epidemiologic follow up to establish the source of infection this information could be relayed to the United States Public Health Service for the application of federal regulations We have not to date had the opportunity of proving the practical effectiveness of our regulation but at least that was our thinking

CHAIRMAN DEAN Dr Herald R Cox Director Viral and Rickettsial Research Lederle Laboratories Division American Cyanamid Company Pearl River New York will now present a paper on the "Chemotherapy of Psittacosis"

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DR HOLDEN May I ask if the rationale behind the use of intermittent therapy is based on experimental work indicating that the virus becomes dependent on aureomycin for growth after a certain interval of time

DR COX Well we have actually done more work experi

It is my belief that if some progress however crude, could be made on this suggestion subsequent research on psittacosis would eventually make much more sense and the validity and significance of the data would be strengthened During lunch today I overheard Dr Huebner make comments similar to the ideas which I have just presented I should like very much to hear again his opinions on this suggestion

DR HUEBNER I think you have covered the matter very well I do not believe that we can hope to be able even to understand each other until we know what the terminology and tests in use mean I don t believe that we are in a position at this time to decide on standard tests I think we shall have to evaluate the tests first and then decide what tests are to be used for isolation and typing of agents and for serologic surveys I do not think that we necessarily need to have the best tests to get such answers Very often in laboratory work we choose a procedure with which we have had a lot of experience It may not be the best tool but our experience with it over a period of several years permits us to interpret it in terms of high order information and a backlog of experience

DR DAVENPORT May I comment on Dr Steele s reference to education as one of the important phases of our future control of the movement of psittacine birds and of human psittacosis I think he is quite right but I think we need as a basis for such education as has been indicated throughout this conference greater laboratory and epidemiologic intelligence I have been impressed that we shall need much more information on the clinical aspects of the various infections produced by the viruses of the psittacosis LGV group and much more information on the laboratory tests which can be applied toward definition of both clinical entity and infection source around which to build public educational programs

Now just a word about the smuggling of psittacine birds Prior to November 1951 we had incorporated the United States Public Health Service Interstate Quarantine Regulations into our rules and regulations for the control of psittacosis With the revision of the United States Public Health Service regulations in November 1951 we were faced with the problem of revising our regulations

ment had no effect on the ability of the birds to transmit the dis ease

Now it seems to me that this is not a valid conclusion I think in that type of study we ought to test whether or not a bird that is treated can transmit the disease to other birds I am not aware of such experiments are you Dr Cox?

DR COX No

DR SABIN Furthermore I think that we ought to try and find out whether or not transmission occurs predominantly from sick birds It is amazing when we trace back the history of cases to find that in so many instances the patients have been exposed to sick birds While an inapparent carrier may under certain con ditions be a transmitter the major portion of transmission may occur from sick birds I think information on bird transmission would be helpful ultimately in the control of the disease it might also influence quarantine measures and should have a bearing on the epidemiology and how much control should actually be at tempted

DELEGATE Might this not be rather similar to instances of prolonged infections when probably the virus is not being shed —at least not in large quantities—but with relapses from time to time a large amount of virus is shed If that is the case it would not seem a very profitable investigation to test birds that had been treated with antibiotic to see whether they were shedders I should presume that any infected bird is a potential shedder and any upset might bring on a relapse and cause it to shed virus in large amounts

DR HUEBNER I think there is another area that needs in vestigation What are the important factors in interhuman spread of psittacosis? It is nearly always the fatal case that is responsible for the spread of psittacosis from man to man That is often true of other diseases It is true of Q fever for example It is very rare for Q fever to be transmitted from one human to another and when this does occur in all cases it is traceable to a fatal or a severe illness

It seems to me that virus dosage must be one of the primary factors affecting spread Furthermore a heavy dosage may be de-

mentally on scrub typhus than we have on psittacosis and unfortunately we have never gotten around to writing this work up But there is no indication whatsoever that any of these agents becomes antibiotic resistant that is resistant to aureomycin at least We did find, though both in psittacosis and scrub typhus, that if we infect the mice and allow them to show signs of illness then treat with antibiotics say on a continuous basis for five to seven days and then terminate antibiotic therapy the mice will look perfectly well when the antibiotic therapy is terminated, but within ten to 14 days they will sicken and die

Now, if we give antibiotic therapy to the point where the mice recover their good health again then wait for the second episode of illness to occur and then resume antibiotic therapy until the mice look well the mice do not sicken and die They are then immune

We have also found that the rate of carriers in the mice is less when the intermittent therapy is used

This procedure is rather logical I believe The growth of the organisms is suppressed by the antibiotic but the organisms are not killed When drug therapy is terminated the organisms start to grow again A sufficient infection is allowed to take place to stimulate the antibody mechanisms When antibiotic therapy is initiated a second time there exists a combination effect of drug therapy plus the antibody mechanism so that the desired results are then obtained

DR HOLDEN My reason for asking the question had to do with the observation that some bacteria become dependent for growth on the presence of antibiotics once resistance is induced

DR COX We have no indication of that in the case of psittacosis

DR SABIN In listening to the discussions on quarantine and therapy it seemed to me that perhaps a distinction has to be made between an infected bird or animal and an animal capable of transmitting the disease In the experiment described by Dr Cox young parakeets were treated with penicillin or aureomycin, but after a certain period when they were tested for the presence of virus the virus was found The conclusion was that the treat

DR COX Insofar as I know it has never been used in any aviary to try to suppress infection

DR HUEBNER All the eggs we buy now are from chickens that have been fed aureomycin

DR COX We do have a problem here but judging from what is known about the epidemiology of the disease I think any bird that is a carrier is potentially a shedder because as Dr Davis has pointed out any form of stress it would seem may make a bird that is apparently healthy become ill and hence a shedder

There are cases of psittacosis on record for instance in which the birds were apparently normal and healthy but when exposed to cold weather they became chilled and developed psittacosis That is an example of a physical condition or stress which causes infection to become manifest Birds also become shedders quite often during the mating season and the period of egg laying although previously they had seemed normal

It looks to me as though we need something more than just a viral static antibiotic We really need something that kills the organisms in order to be safe So far I know of no antibiotic which really sterilizes the tissues of the host

We have a similar situation in man with Brill s Disease People who have apparently recovered from typhus and have been normal for many years for some unknown reason perhaps a physiologic breakdown suffer a reoccurrence of the same disease

I am not sure but I think that the only way the problem can be solved is to find an antibiotic that actually sterilizes Whether the individual would be left in an immune state under those conditions would be hard to say We just do not know

DR IRONS I might say that in this turkey problem in Texas that was the thing that aroused our curiosity because we were dealing with a processing plant in which there were three epidemics This same firm also operated a big feed processing plant one of the first in the area to add aureomycin to feeds Since it financed the operation of many of the turkey ranchers—hens eggs and feed bills—we wondered if perhaps in those flocks with the infection some of the younger birds that would have died had it not been for the use of the antibiotic feeds got to market and

fined by access to large amounts of the agent in a confined or closed air space Thus pigeons and other outdoor birds may not be very important transmitters compared to sick parrots or other household birds

DR SHAUGHNESSY Another question that should be given consideration is the relative potency or toxigenicity if you will of the virus which is shed by parrots or other imported birds and that shed by birds bred in this country It is assumed that the drop in the human fatality rate that we have seen recently is due entirely to the use of antibiotics It may be that that is due in part to differences in the source of the infection

DR DAVIS I think that probably in this whole meeting here we have neglected an extremely important facet of the problem namely the epidemiology of the disease of psittacine origin There is a good deal of epidemiologic evidence on this very important point that Dr Sabin brought out

The sick psittacine birds are probably the most important source of disease Now unfortunately we do not know just why it is that psittacine birds which apparently carry the virus for long periods become ill and then obviously shed the virus There are a number of records of birds which were isolated in households for periods of months or years and suddenly they became ill and were a source of disease in humans I think it is also interesting that the first isolations of psittacosis virus from pigeons by Pinkerton and Swank were made from pigeons which were thiamine deficient

DR SPRUNT One thing which I believe has been overlooked is the spread of the disease by sick birds and its spread by a well carrier Since a sick bird is probably shedding virus at all times it is easily understood how a person close to the bird gets the infection Our present understanding of the sick bird is not our main concern The danger as I see it is from the apparently healthy bird which does not shed the virus but may infect the personnel who prepare it for the food industry

DR HUNTER Has aureomycin ever been used in treating the disease in sick birds or as a feed supplement as is done in chicken feeding?

DR COX I think under certain circumstances it is possible to sterilize the tissues but these circumstances are rather rare and not what you might call practical conditions

Experimentally in the laboratory with most of the rickettsias for instance if guinea pigs are infected and therapy started within 24 hours the infection is suppressed but engenders no immune response But this is not what you encounter clinically in the field From the practical standpoint these antibiotics do not sterilize It just happens that a laboratory can be prepared because it expects an infection and starts therapy within 12 to 24 hours I think that that ordinarily does not occur Generally the physician does not see a patient until he has been sick for 48 or 72 hours In that case the antibiotic does not sterilize

CHAIRMAN DEAN The discussion has brought this question to my mind What does the future hold and where do we go from here? I don t know whether that is a proper subject to introduce into the discussion or not but I wonder if anyone has any thoughts along that line

DR SUSSMAN Mr Chairman perhaps this resolution might help It was prepared by a group of the men here and I should like to offer it to the conference

WHEREAS This Symposium on Psittacosis has made possible the presentation of a very fine and complete review of the presently available scientific knowledge and lack of knowledge concerning the epizootiology and epidemiology of this disease and

WHEREAS This Symposium was definitely needed in order to point up the needs and the issues involved in the control of the disease in birds and man therefore be it

Resolved That those in attendance do commend

First the New Jersey Agricultural Experiment Station at Rutgers University and in particular Dean W H Martin and Dr F R Beaudette for their interest and their conviction of the needs for this Symposium and their action in organizing same and

Second the Hartz Mountain Products Company for their efforts and graciousness as co-hosts with Rutgers University and their attitude desiring unfettered unhampered unrestricted free and open discussion of the problems that affect the pet bird industry

were carriers I have no evidence for that one way or the other

Another thing that is interesting is what Dr Cox said regarding the need for an agent to sterilize the tissues I believe we probably do have in these antibiotics a method of sterilizing the tissues We noticed as these epidemics came along that there was a larger and larger percentage of cases in which it was impossible apparently, to confirm the diagnosis serologically When we checked the records we found a very high correlation of poor serologic re sponse or none at all with the institution of early treatment This was quite pronounced—it was most pronounced with two labora tory acquired infections I felt very strongly that both these peo ple must have turkey psittacosis—they were working with prac tically nothing else but the turkey ornithosis virus and rabies virus —and we had the aureomycin all ready for them in advance know ing just as Dr Delaplane did that they would get infected

We gave the antibiotic to them so early (one of them got treat ment within 12 hours and the other within 24 hours after onset) that the fever in both promptly subsided within 24 to 36 hours As we did not succeed in recovering the virus from the throat washings or the blood of these two patients before treatment was started and as neither of them later developed any titer none whatsoever we had no laboratory evidence to support a diagnosis of psittacosis and yet I am absolutely certain that that was the proper diagnosis

So I believe if the treatment is started early enough it is pos sible to kill off the virus I suppose too that people probably have no immunity with this type of experience

Might I say also that the physicians who treated these pa tients tried quite a variety of combinations and an interesting thing was that we had only one death and that was in a hospital ized patient who reasonably could have been expected to recover The Negro physician who treated this man fully believes that the poor response was due to the fact that he gave both aureomycin and penicillin to this patient

Generally speaking aureomycin or terramycin was used and we believe there is very good evidence that good response is ob tained with both In some few cases chloromycetin chloroam phenicol was used in combination with terramycin or aureomycin

ion of their work by other workers in the same field Too often *when a paper is presented to an audience many of those who* listen to it have never done any work in that particular field and they are not in a position to offer a critical opinion but here we have a group of specialists any one of whom is capable of giving a good criticism

I think the conference has brought out the fact too that certain problems should be re investigated Certain things that we have taken for granted in the past would perhaps not suffer on re-examination

As a poultry pathologist I am impressed with one thing and that is that we still lack a simple and accurate method of diagnosis

Now I came here with the idea that perhaps if I were presented with a parrot and if after excluding the other virus diseases with which I am familiar I found that this parrot showed the gross pathology of what we accept as psittacosis and I had isolated an infectious agent from it in all likelihood the agent would be psittacosis But after listening to these several papers I go away from here with the idea that if anybody presents a parrot to me and I chance to isolate an agent from it that is not Newcastle virus or something else I wont know whether the parrot died from lice or leprosy!

I think that there is a very great need for a simple and yet reliable method of identification

If we are ever going to know a great deal about this disease we shall have to know the extent of infection and I think that those who are working in this field should interest the poultry pathologists There are about 200 or 250 poultry pathologists in this country and to them come a very large number of birds each year These men have certain facilities which could be modified or used for the diagnosis of this infection and if some standard procedure—standard if possible—could be recommended to them I am sure that we would get a much better picture of the incidence of this disease

A good many years ago certain states began a pullorum disease eradication program This is an infection that is transmitted through the egg and carrier birds can be detected by means of

and the public health and we particularly desire to thank Mr Gustav Stern Mr Max Stern and Mr William Odenwald and

Further this Symposium recommends the establishment of a committee under the sponsorship of Rutgers University to elaborate on the future studies needed in this field and to present such needs to appropriate industry and governmental agencies in order to have such work carried forward.

CHAIRMAN DEAN Do you make that as a resolution for the adoption of the group here Dr Sussman?

DR SUSSMAN Yes I do

(Motion seconded by Dr Richard E Shope vote taken motion carried unanimously)

CHAIRMAN DEAN I think at this point it would be proper for the Chairman to turn the meeting over to Dr Beaudette to see if he has any announcements or any further business to bring before the conference

DR BEAUDETTE First off I personally want to thank every one of you for attending our conference I know it meant a sacrifice of time on your part And double thanks are due those who spent a tremendous amount of time in preparing these splendid papers We appreciate that very much

Now Mr Stern asked me to say that because he has to get ready for an around the world trip which begins tomorrow he has been unable to be here to see the end of this conference but he too wants to add his thanks

Now I have been asked by three or four people about the purpose of this conference Mr Stern and I discussed that and I do not suppose either of us could put it into words any better than those already stated

As you know we intend to publish these papers and discussions If we do no more than that I am sure that the meeting will have justified itself But I think the meeting has had other effects too I am sure that there has been some new material presented here that would not otherwise be available I feel too that it has afforded an opportunity for research workers to get a critical opin

this is only the beginning and that if anyone has any ideas about needed research—that might come out of this committee perhaps —he still has a dollar or two left

And I will end up by wishing you all a very pleasant trip home Thank you again

an agglutination test But when states worked independently it became evident that we did not have standard methods This was pointed out when a certain state, after testing about 20 000 birds had not found a single reactor and consequently got suspicious of the results It was reported that this worker was making his antigen with a soil organism I can t believe it was that bad but apparently he had used some organism other than *Salmonella pullorum*

At any rate as the result of that, we formed an organization known as the Northeastern Conference of Laboratory Workers in Pullorum Disease Control The conference appointed an Antigen Committee among others and standardized procedures so that if a chicken passes the test in Massachusetts it will also pass the test in Connecticut New Jersey, or any other state With standardized procedures we could enlist the help of poultry pathologists and eventually get a very good picture of the incidence of this particular infection

Now this resolution that has been passed pleases me immensely that is certain phases of it because it sets up the machinery whereby something constructive can be accomplished

Had it not been for that resolution I could only have said that Mr Stern did not intend this to be just a gathering to discuss psittacosis and perhaps enjoy a pleasant association but to be a means of renewing interest in a timely problem However we did not know where to go from here because this is not an official organization We have no authority of any kind and the machinery for setting up anything for the future was simply lacking I am very pleased Dr Sussman for the resolution that has been passed and I hope that something will come out of it

I think it would be well if we could have a central laboratory or perhaps several appropriately located laboratories in different parts of the country to which poultry pathologists could send specimens for an accurate diagnosis The laboratories could be patterned after the *Salmonella* laboratories We regularly isolate *Salmonella* organisms but when we wish to have them typed we send them to Atlanta I think something along that line for psittacosis would be a very good thing

And then my last word is that Mr Stern asked me to say that

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